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(12) **United States Patent**
**Humayun et al.**

(10) **Patent No.: US 8,808,687 B2**
(45) **Date of Patent: Aug. 19, 2014**

(54) **BIOCOMPATIBLE SUBSTRATE FOR FACILITATING INTERCONNECTIONS BETWEEN STEM CELLS AND TARGET TISSUES AND METHODS FOR IMPLANTING SAME**

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( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 82 days.

(21) Appl. No.: **13/181,279**

(22) Filed: **Jul. 12, 2011**

(65) **Prior Publication Data**

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**Related U.S. Application Data**

(60) Provisional application No. 61/363,630, filed on Jul. 12, 2010, provisional application No. 61/481,004, filed on Apr. 29, 2011, provisional application No. 61/387,830, filed on Sep. 29, 2010.

(51) **Int. Cl.**

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| ***A61F 2/02*** | (2006.01) |
| ***A61K 35/30*** | (2006.01) |
| ***B32B 3/00*** | (2006.01) |
| ***A61K 47/34*** | (2006.01) |
| ***A61K 9/00*** | (2006.01) |
| ***A61L 27/38*** | (2006.01) |

(52) **U.S. Cl.**

CPC ............... ***A61K 9/0051*** (2013.01); ***A61K 47/34*** (2013.01); *A61L 2430/16* (2013.01); ***A61L 27/3813*** (2013.01); ***A61K 35/30*** (2013.01); ***A61L 27/3834*** (2013.01)

USPC ........................ **424/93.7**; 623/23.72; 428/156

(58) **Field of Classification Search**

CPC .......... A61F 2/14; C12N 5/0621; C12N 5/00; A61K 9/00; A12M 3/00

See application file for complete search history.

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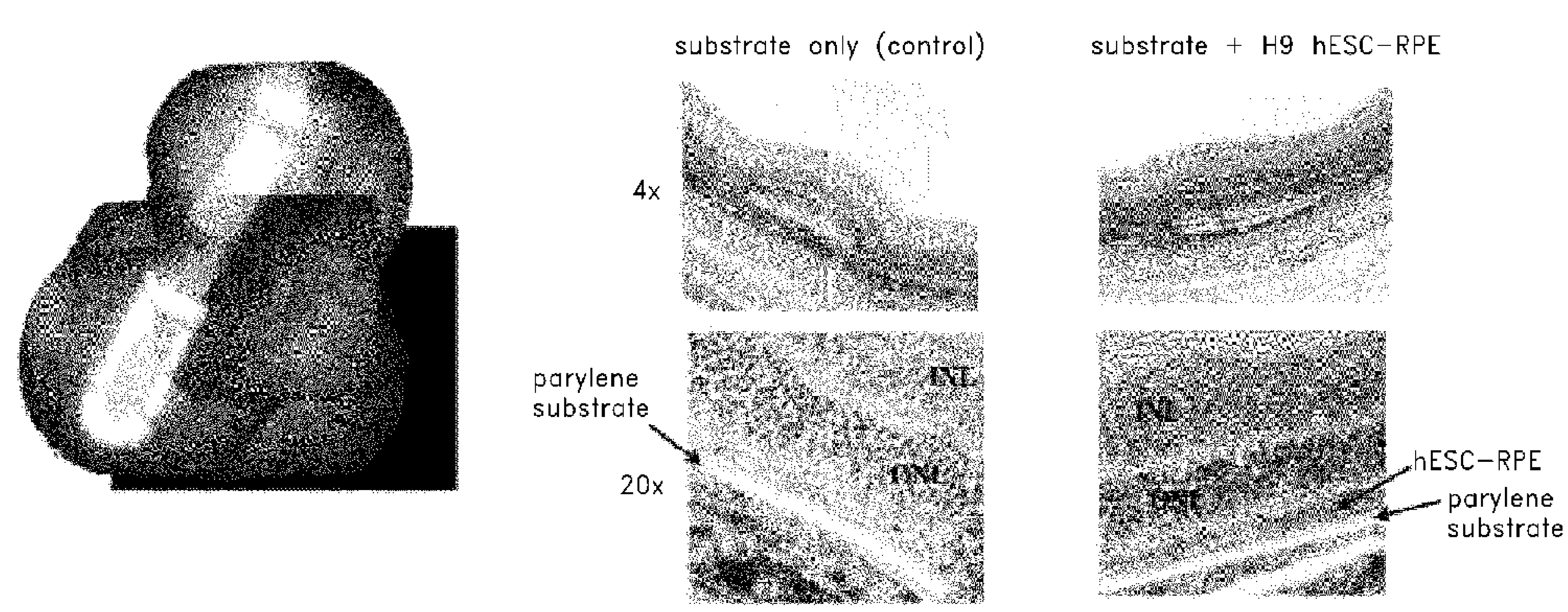
*Assistant Examiner* — Trent R Clarke
(74) *Attorney, Agent, or Firm* — Knobbe Martens Olson & Bear, LLP

(57) **ABSTRACT**

Disclosed herein are substrates for cell delivery to target tissues requiring treatment for various diseases that induce cell death, damage or loss of function. The substrates are configured to provide seeded cells, including stem cells, with a structural support that allows interconnection with and transmission of biological signals between the cells and the target tissue.

**24 Claims, 33 Drawing Sheets**



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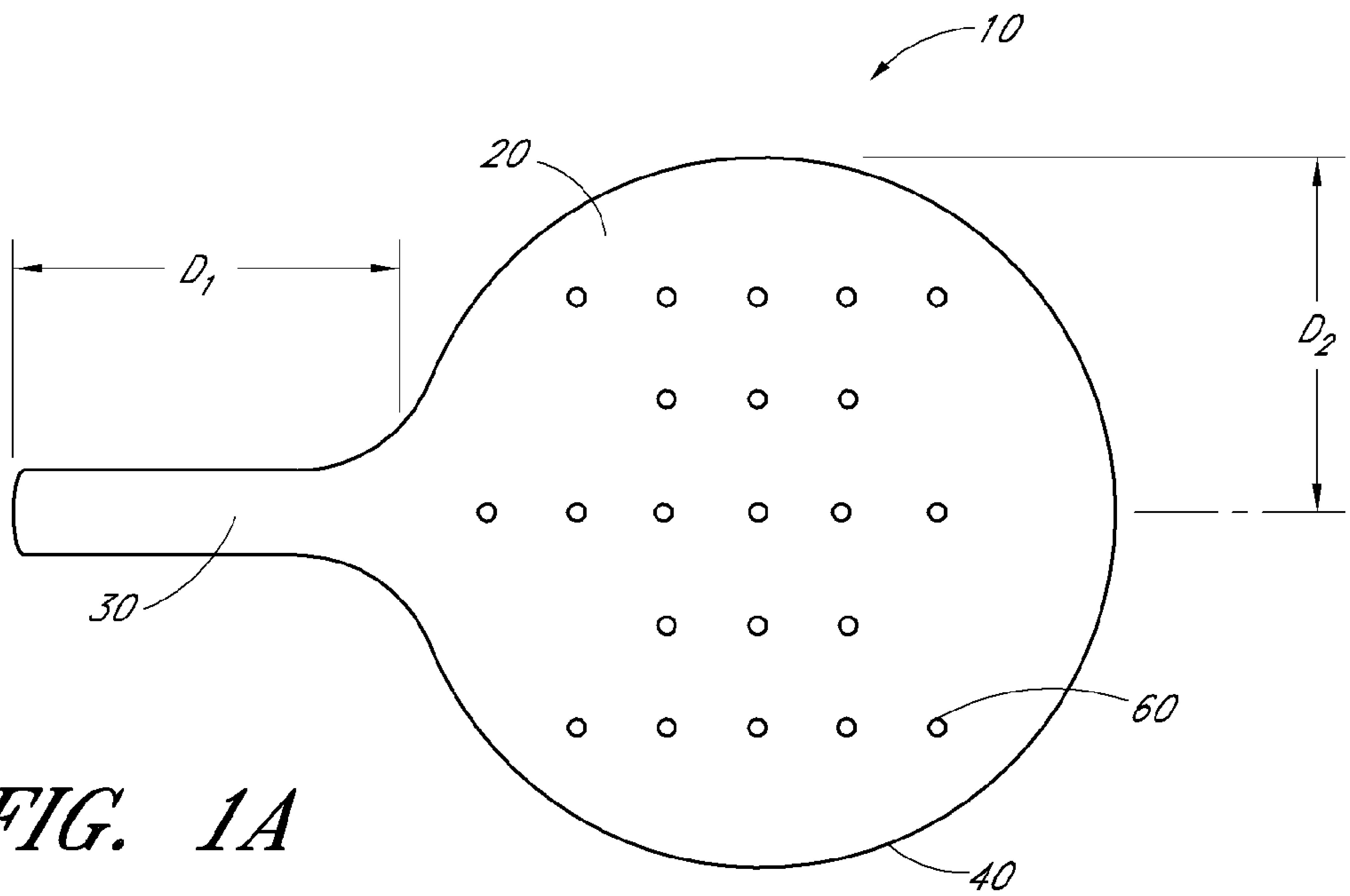


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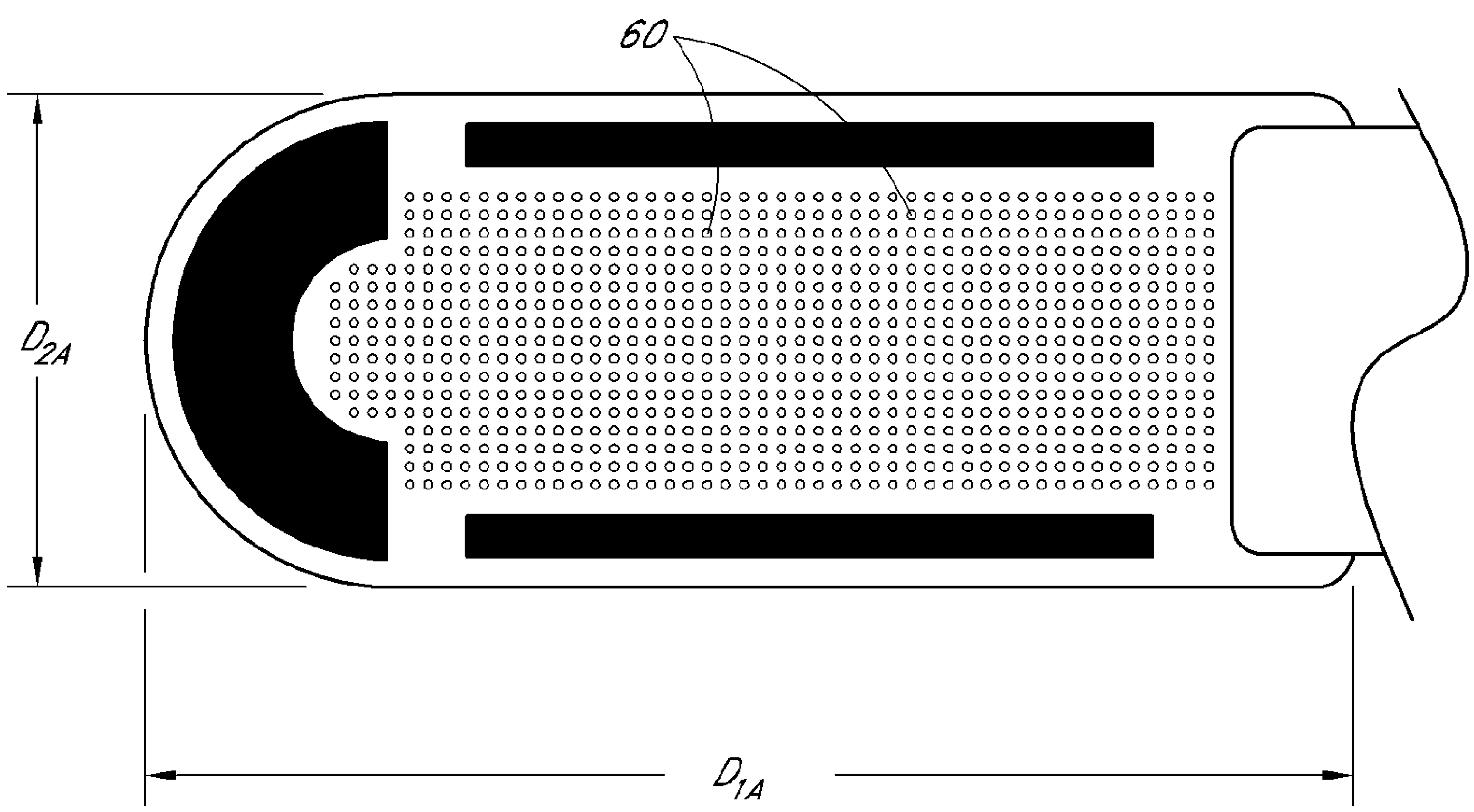


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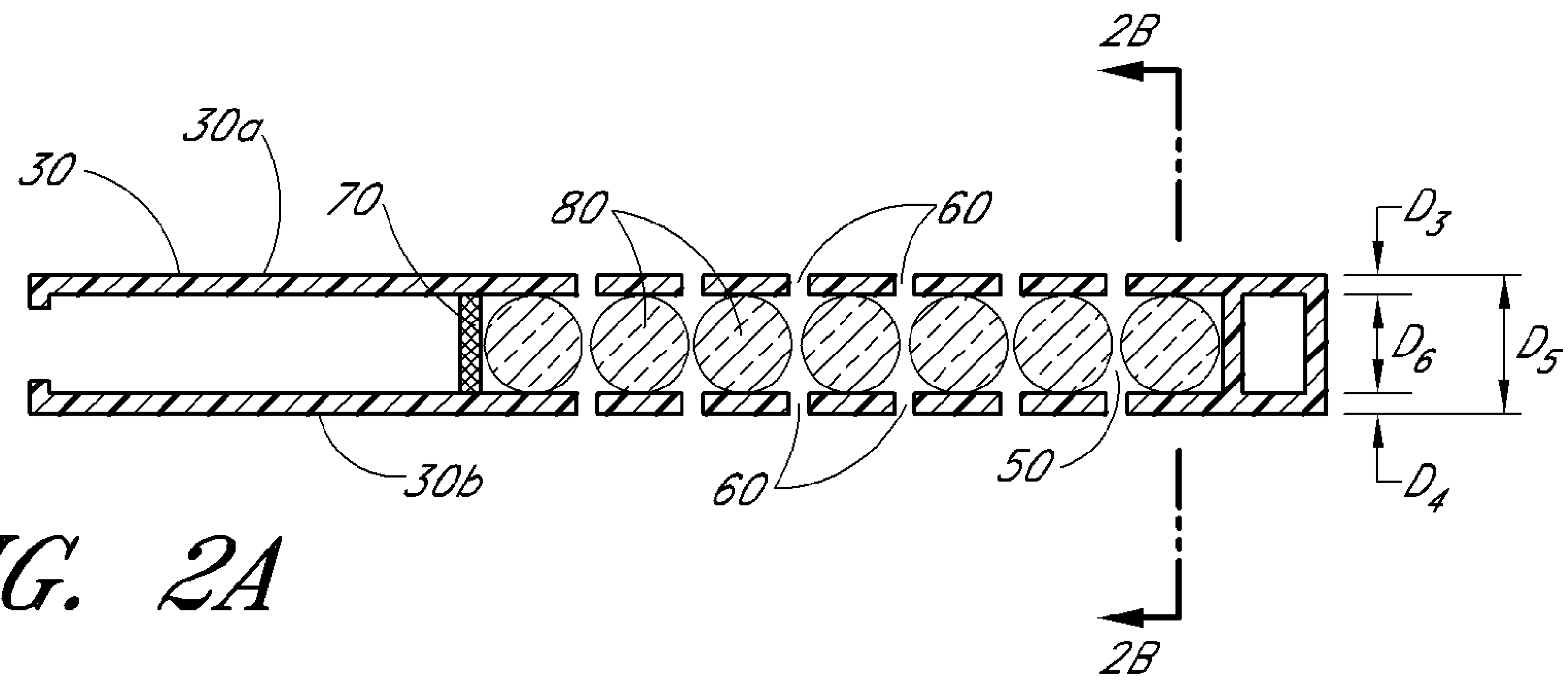


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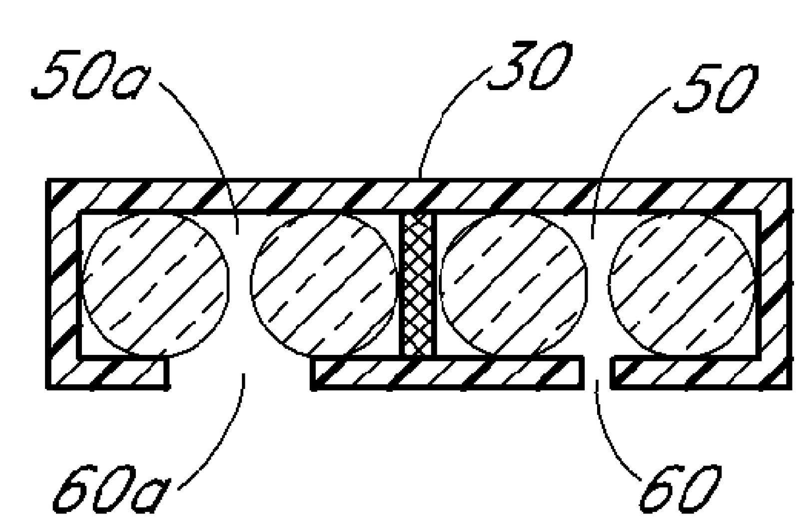


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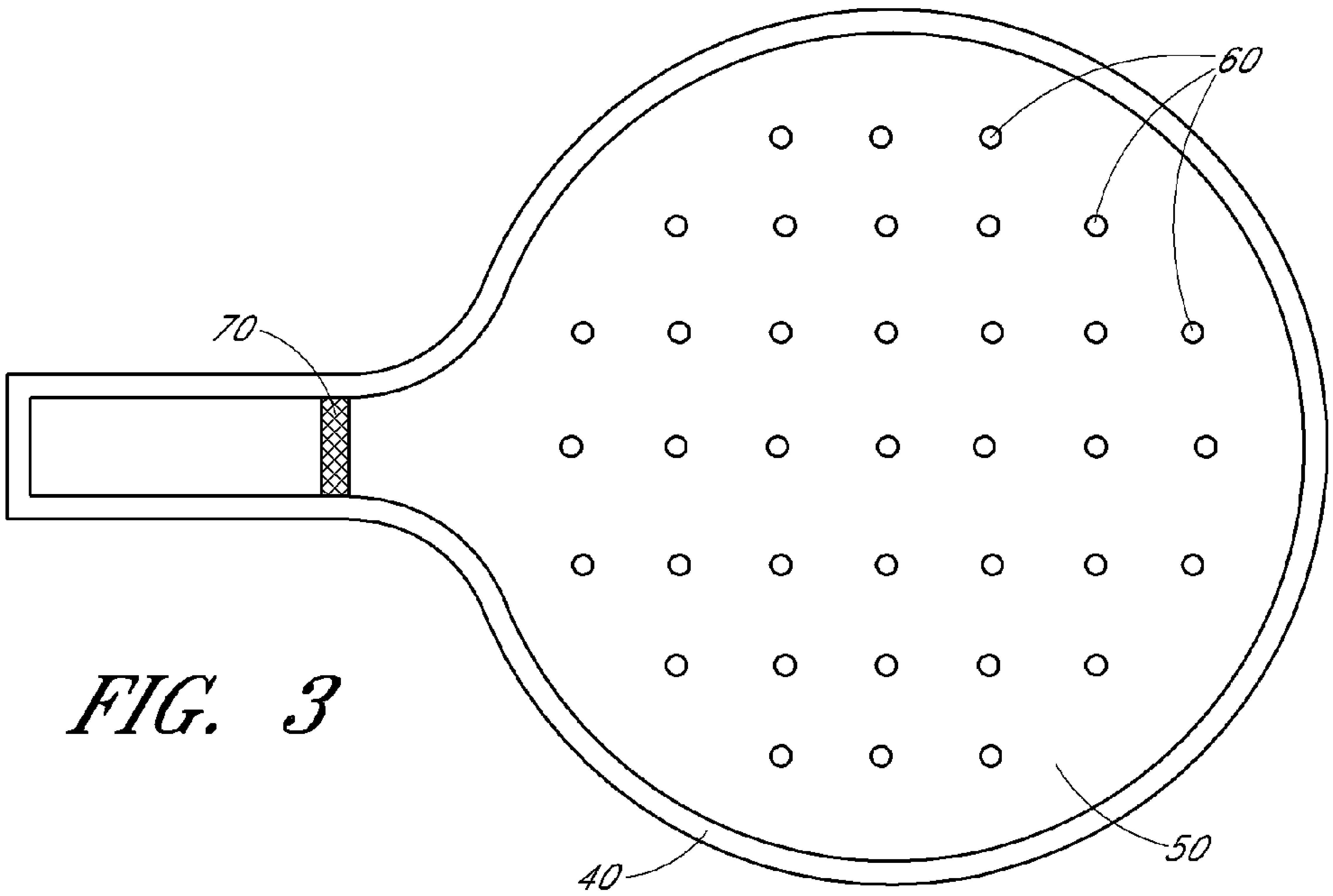
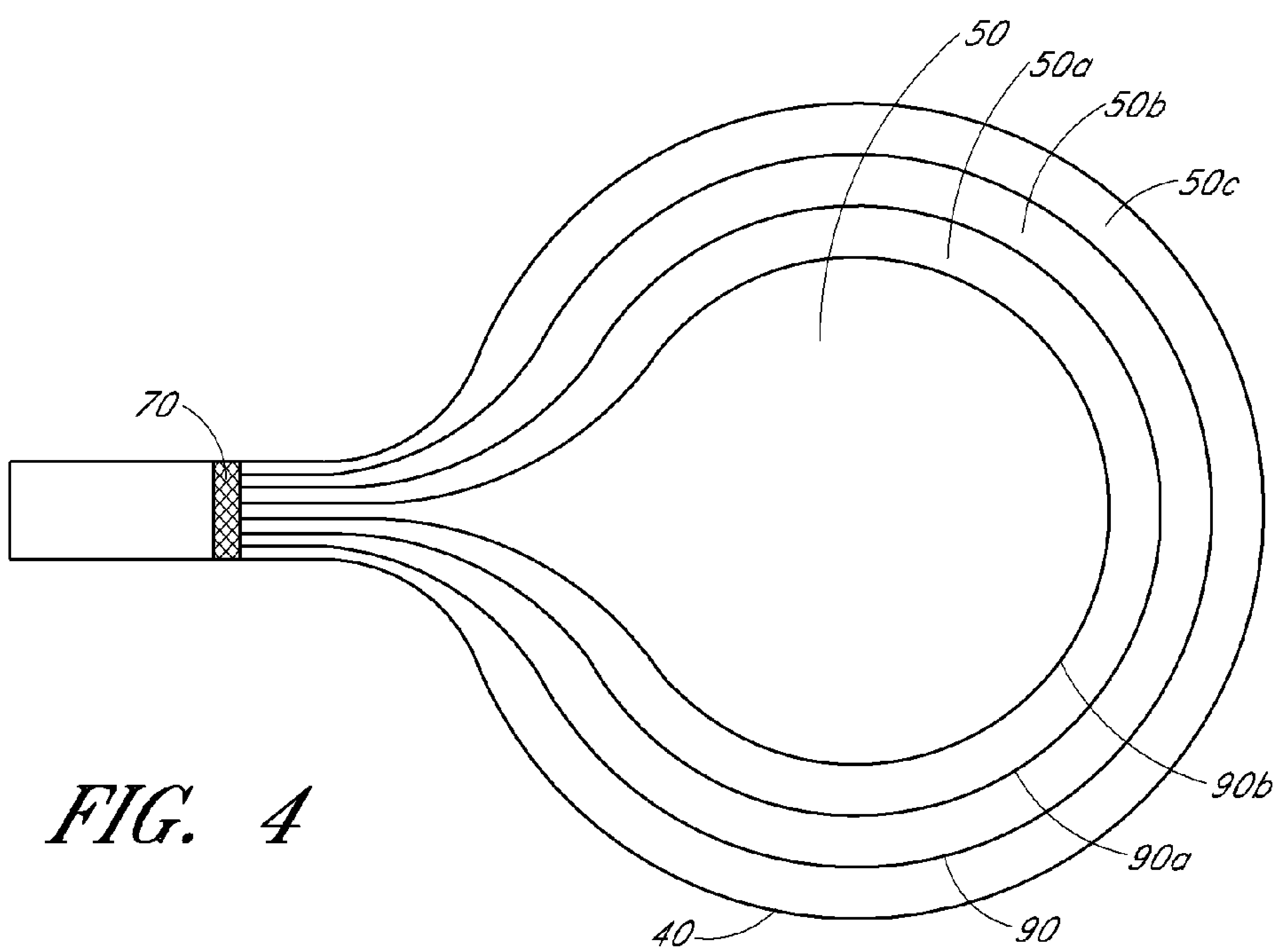



FIG. 3

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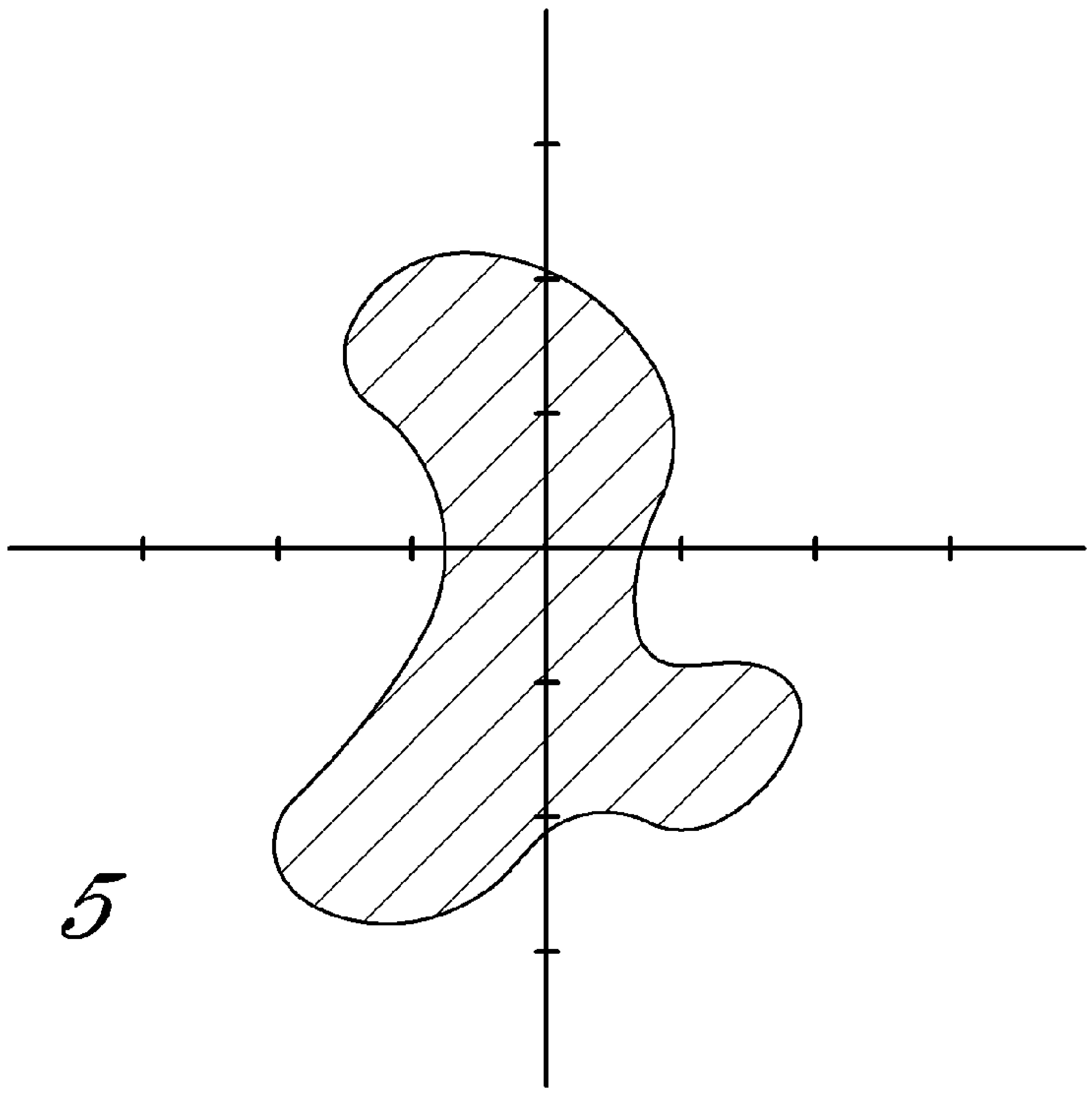
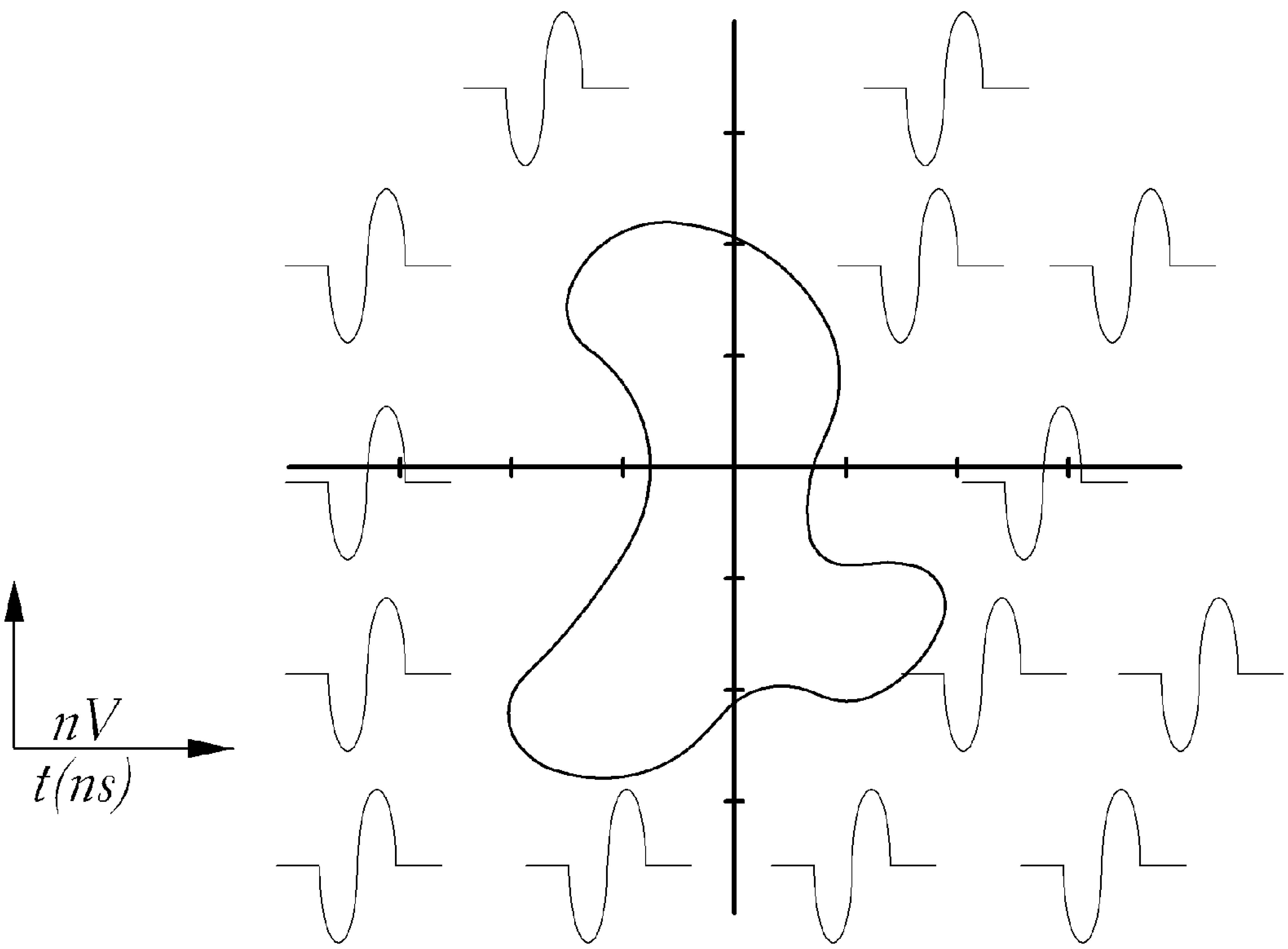


FIG. 5

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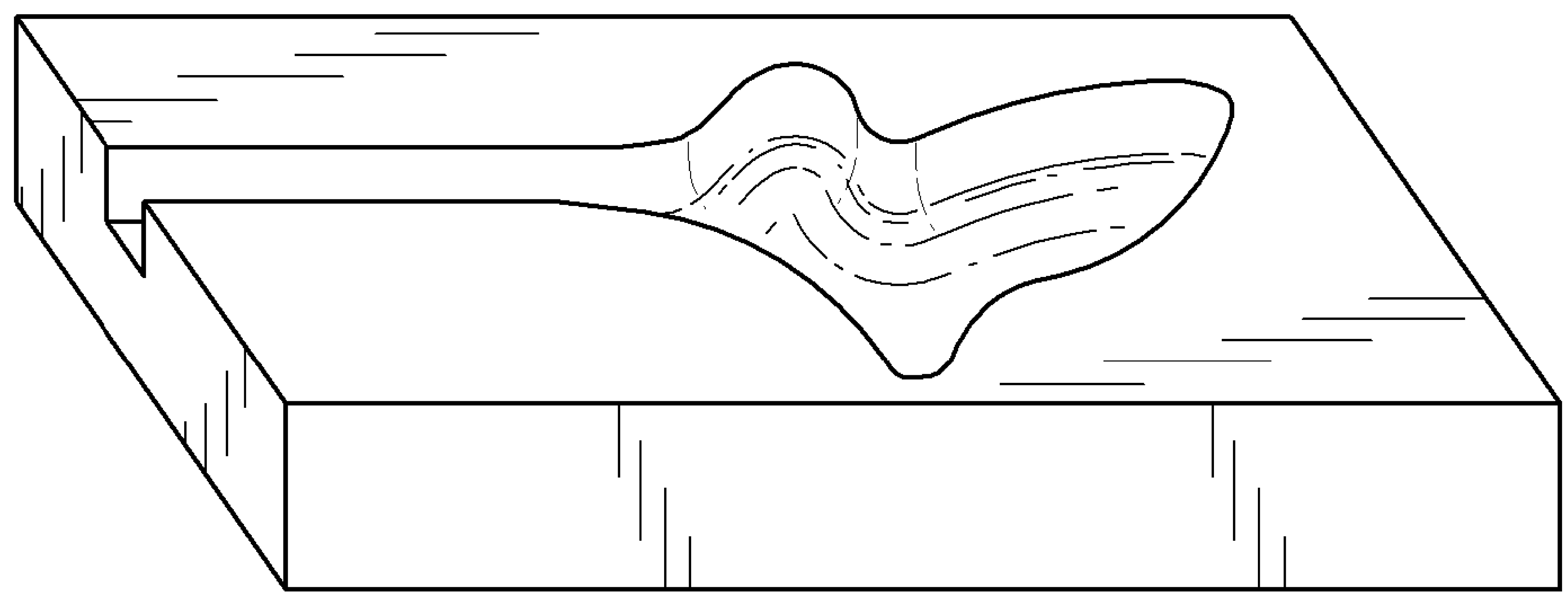


FIG. 7

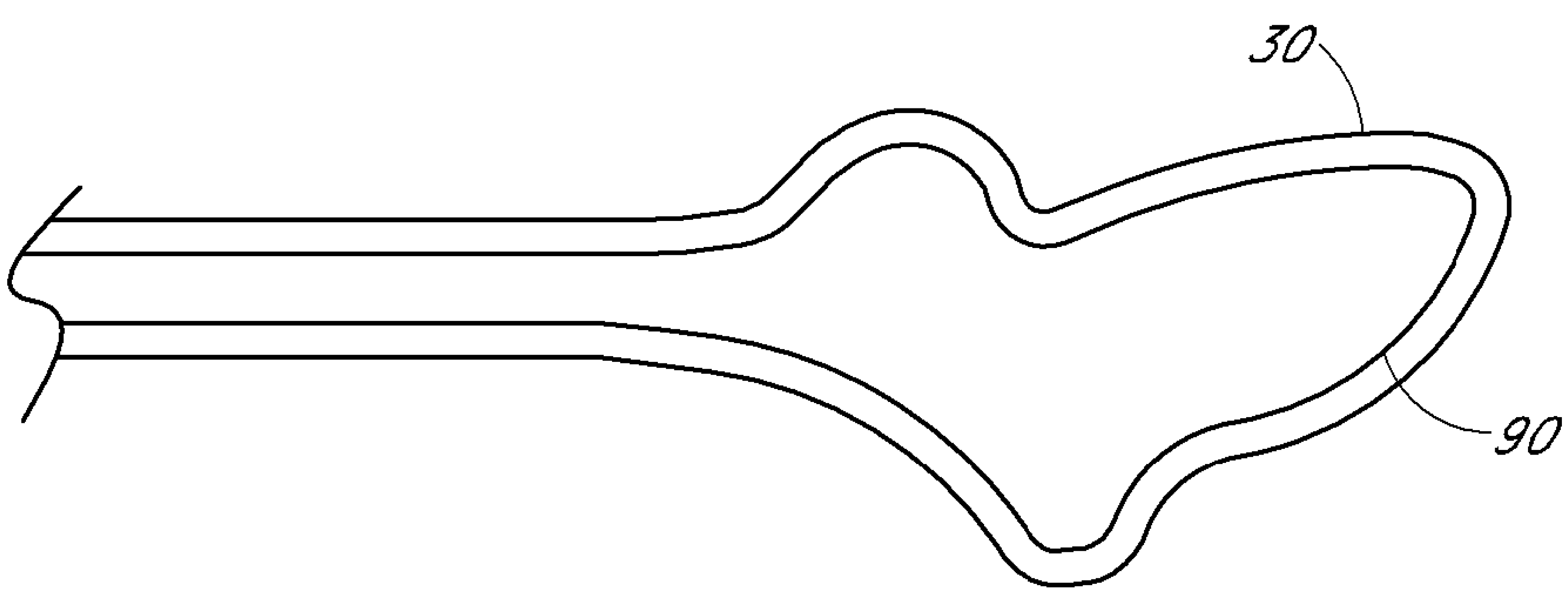



FIG. 8

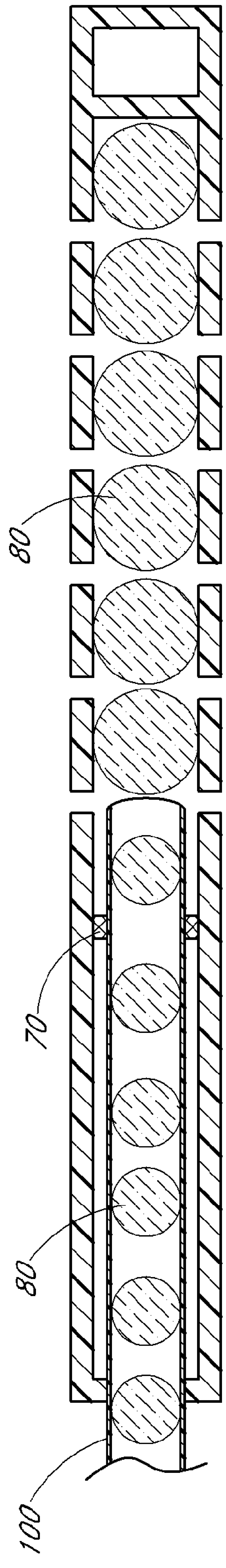



FIG. 9

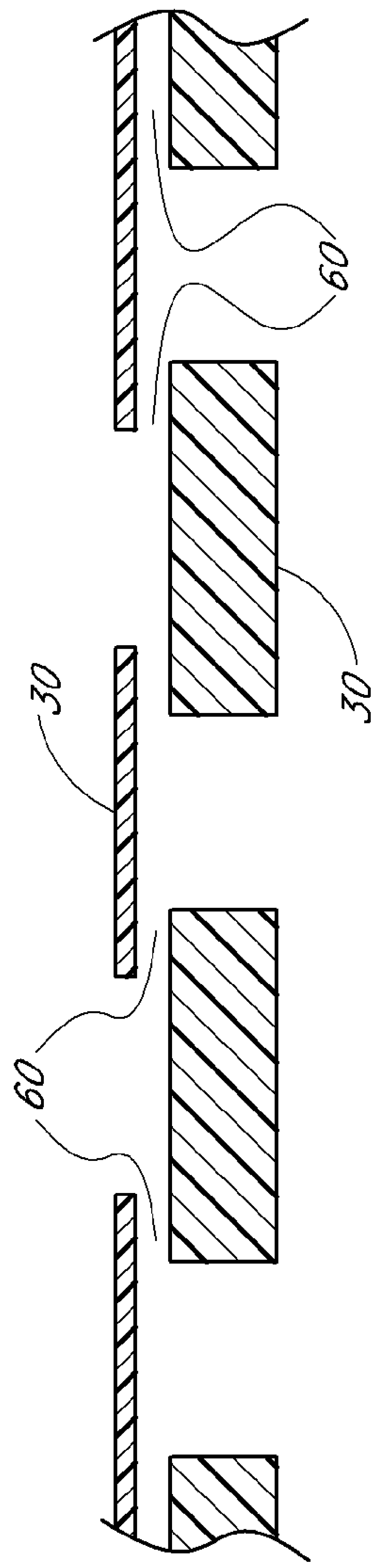



FIG. 10

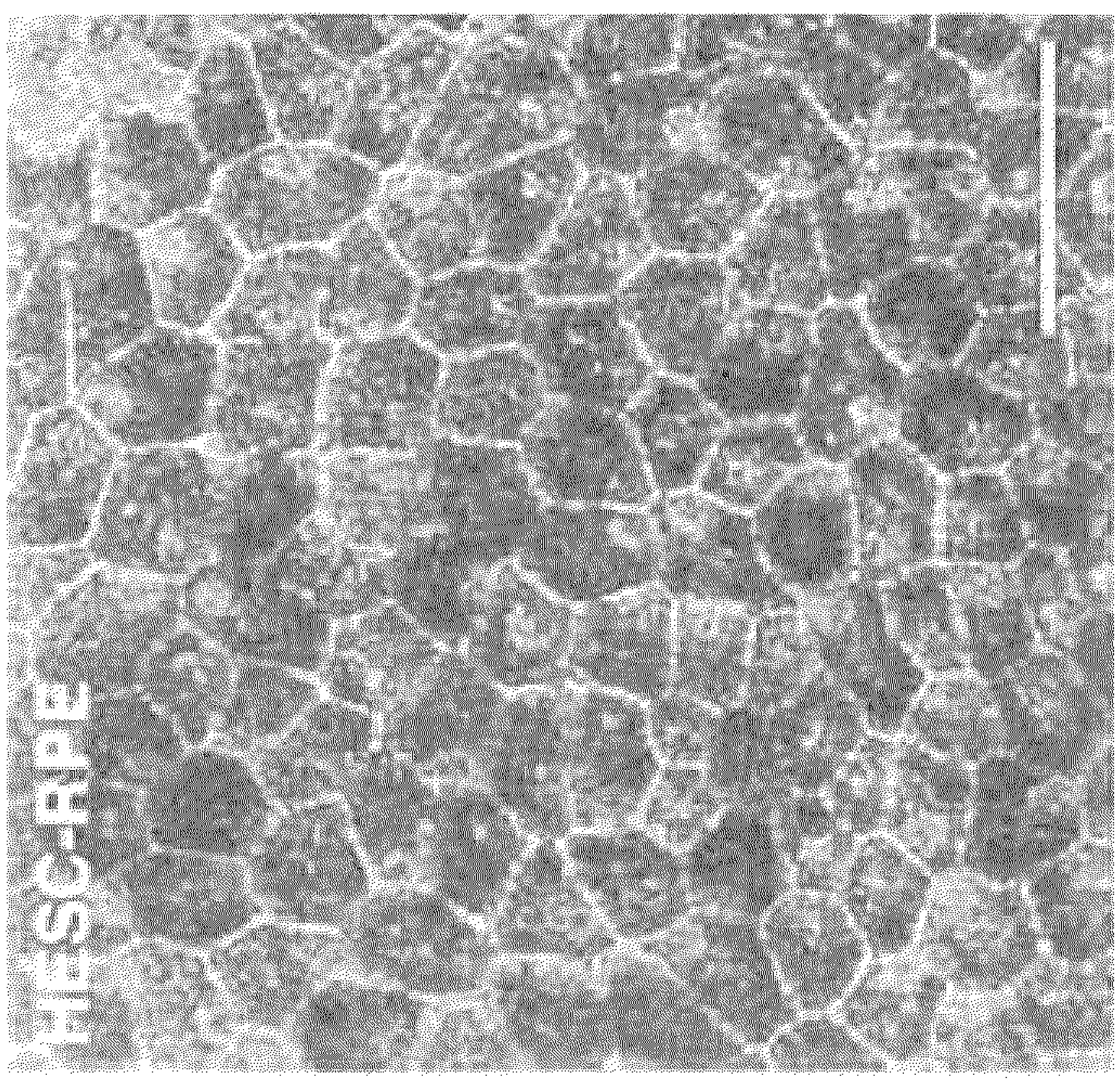



*FIG. 11A*

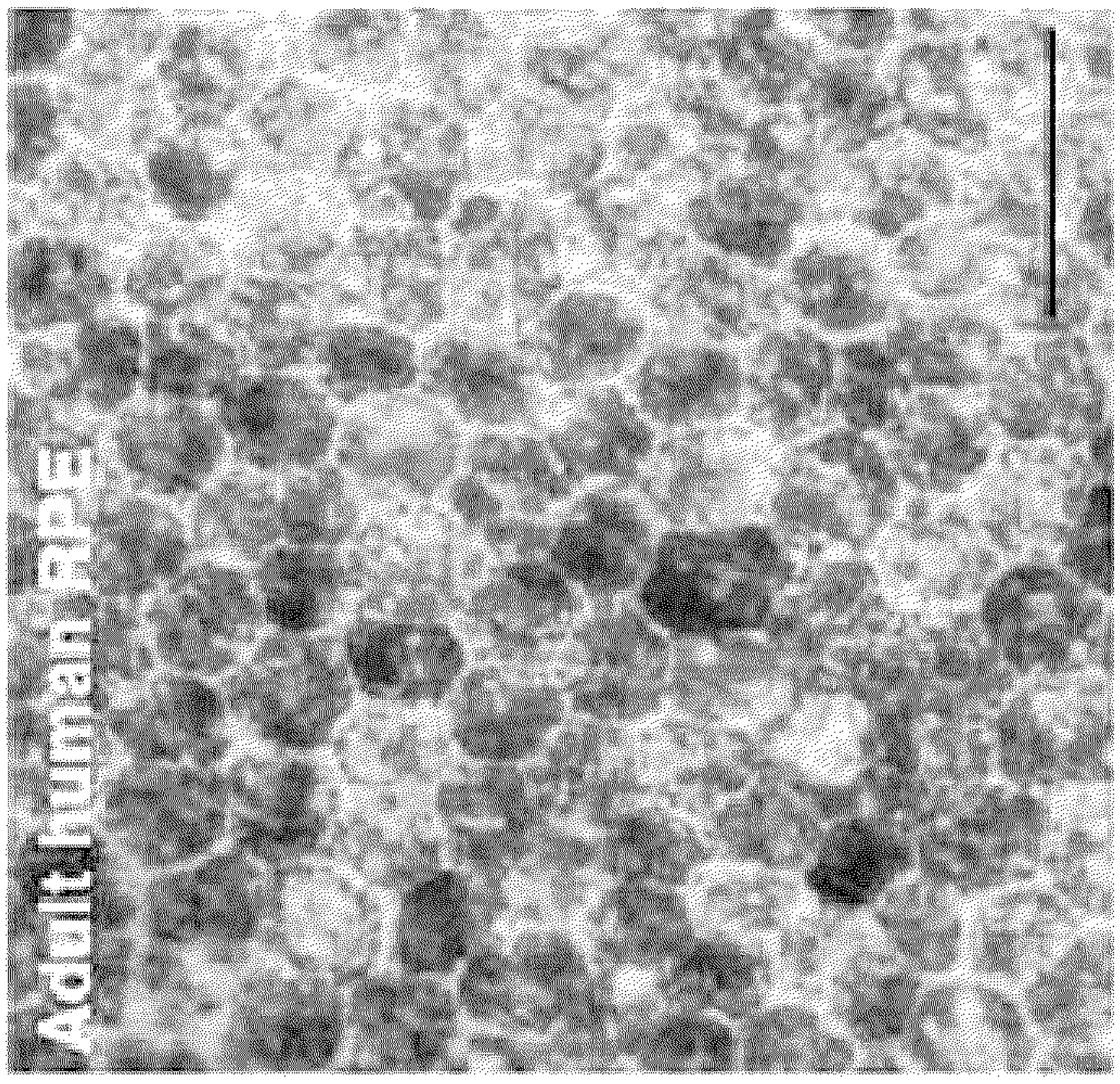



*FIG. 11B*

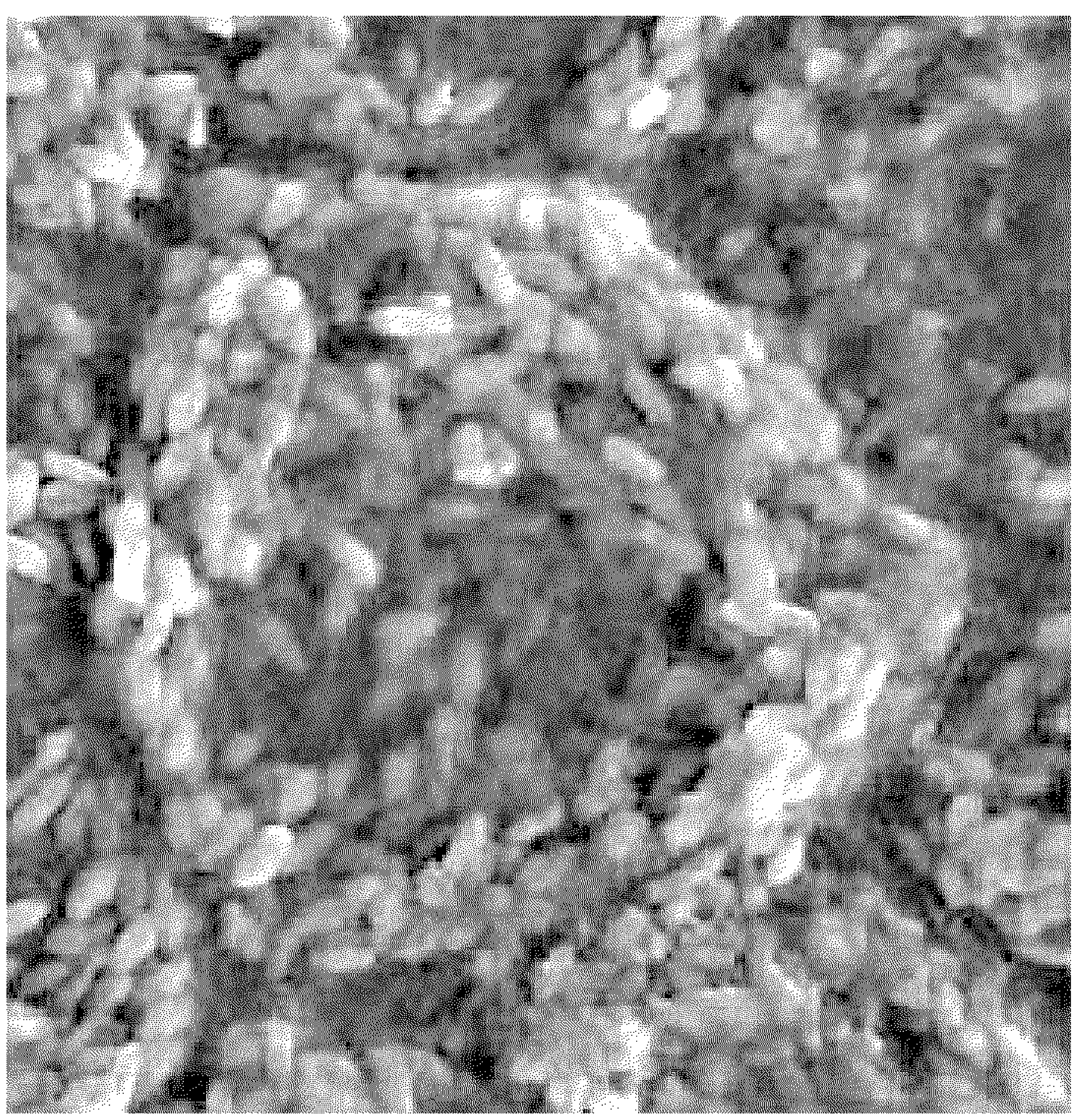


FIG. 12A

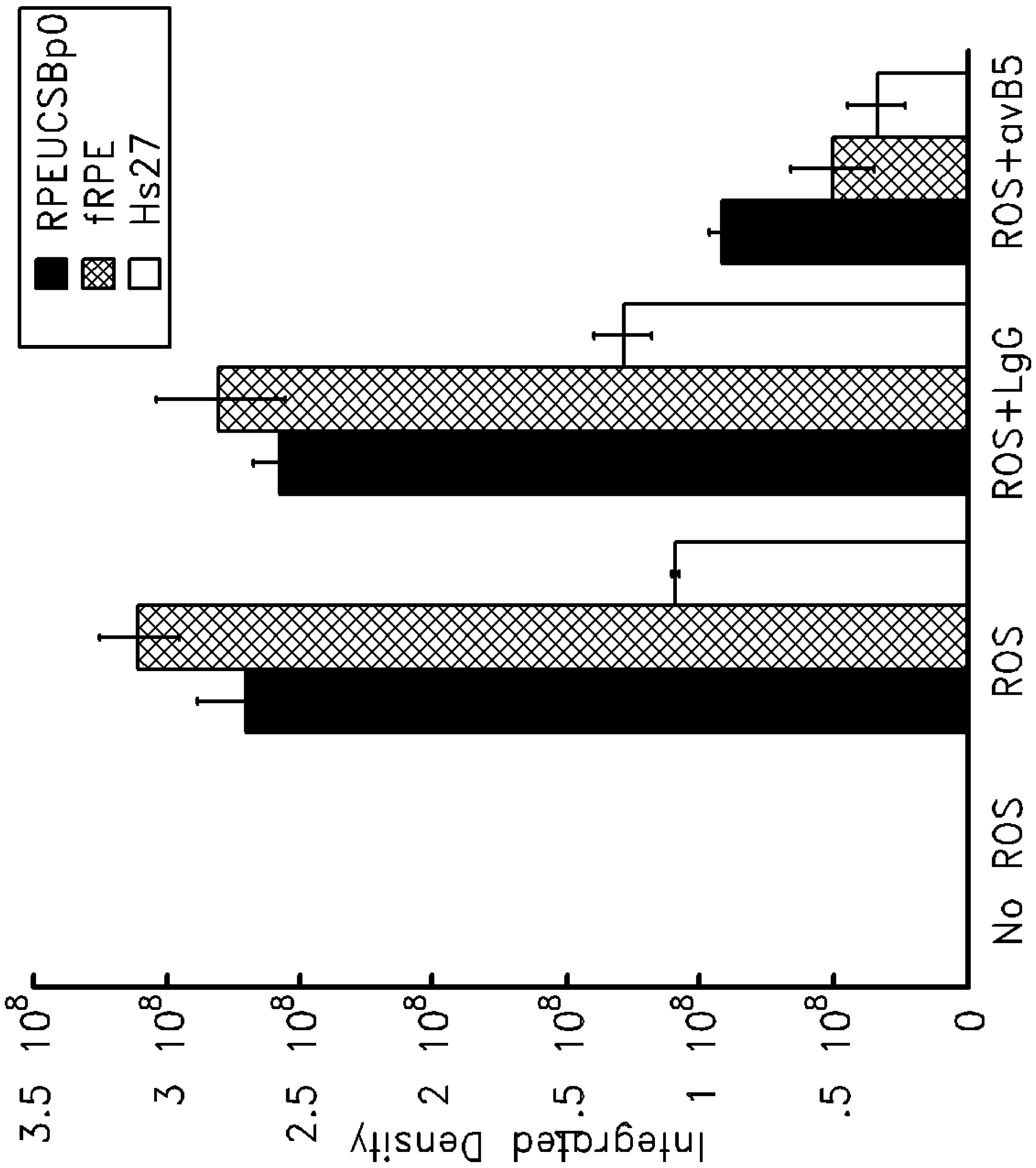



FIG. 12B

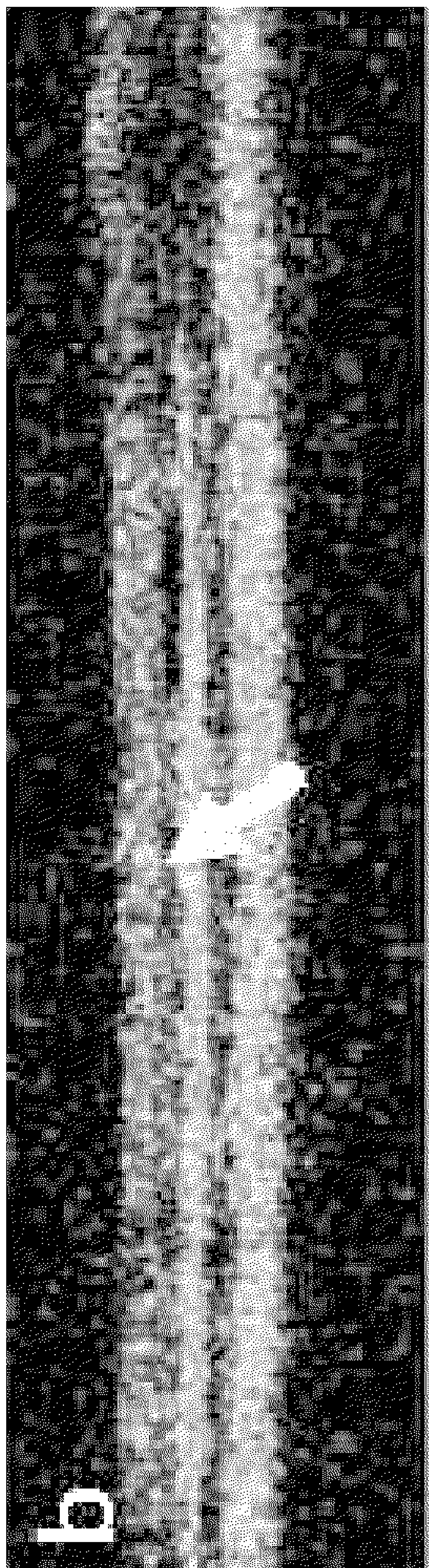



*FIG. 13B*

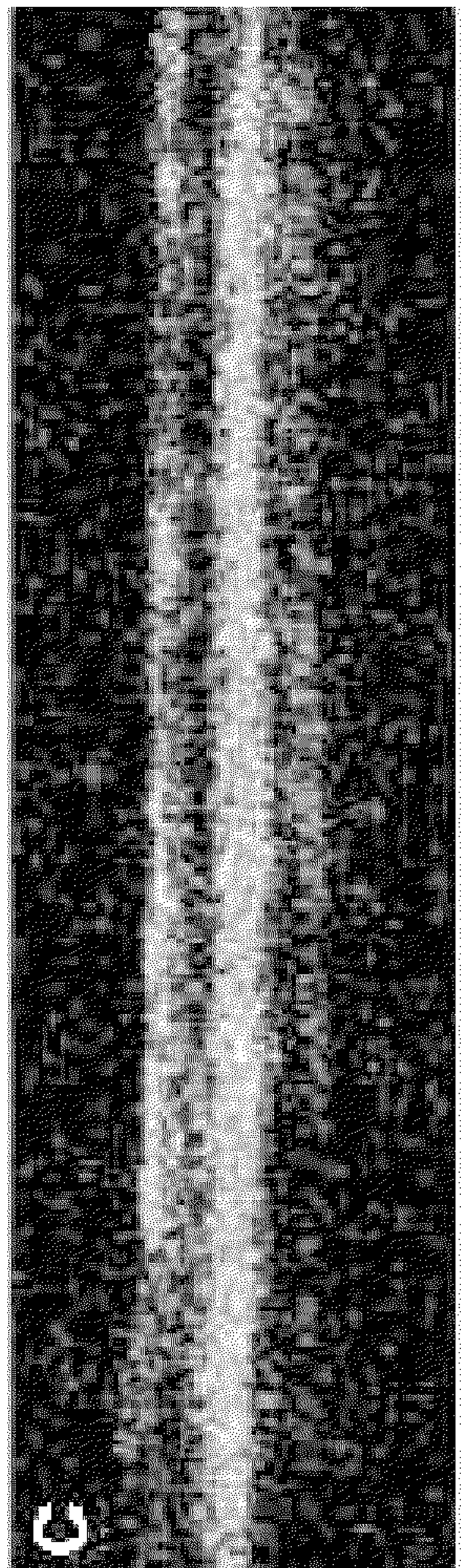



*FIG. 13C*

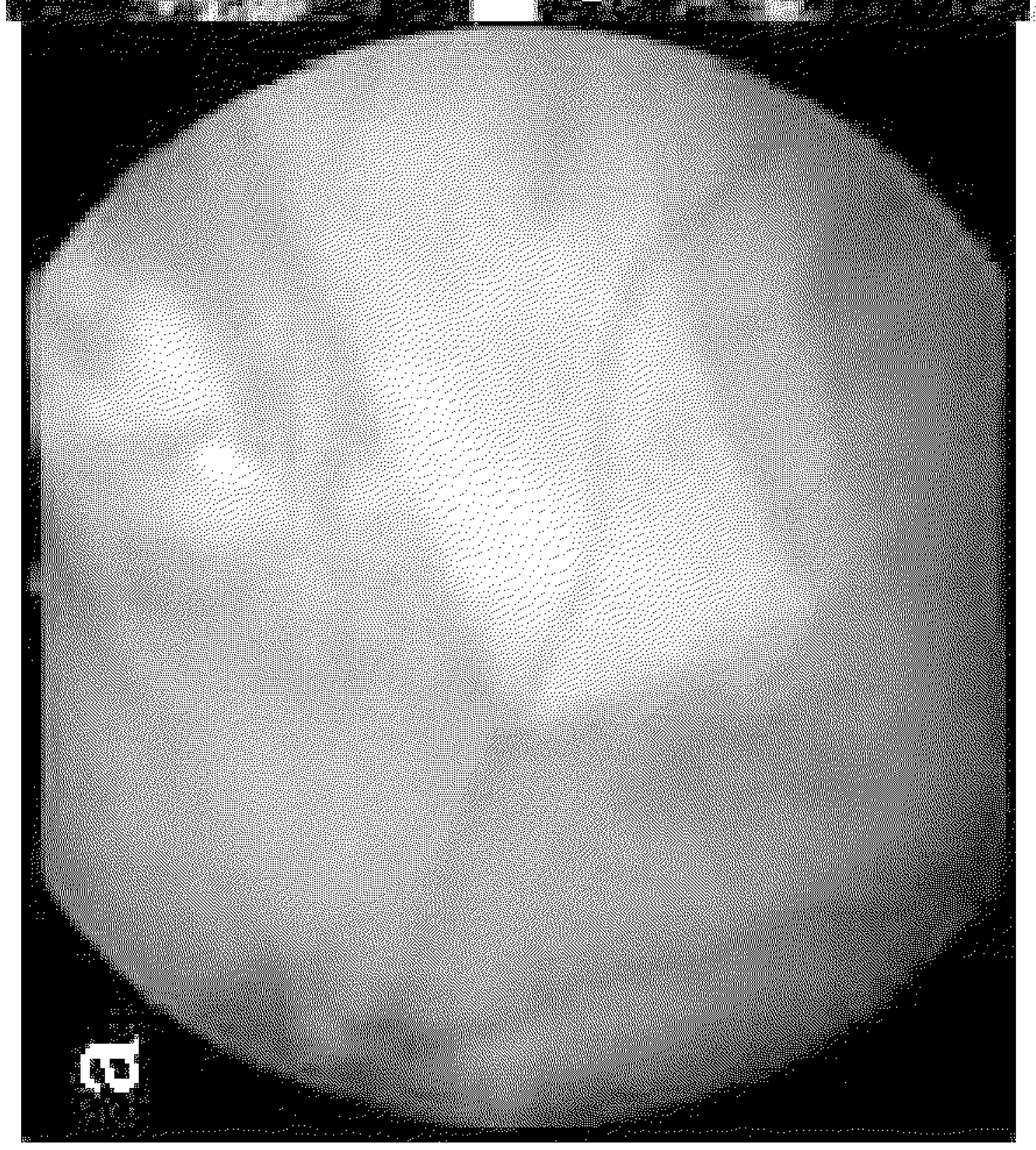



*FIG. 13A*

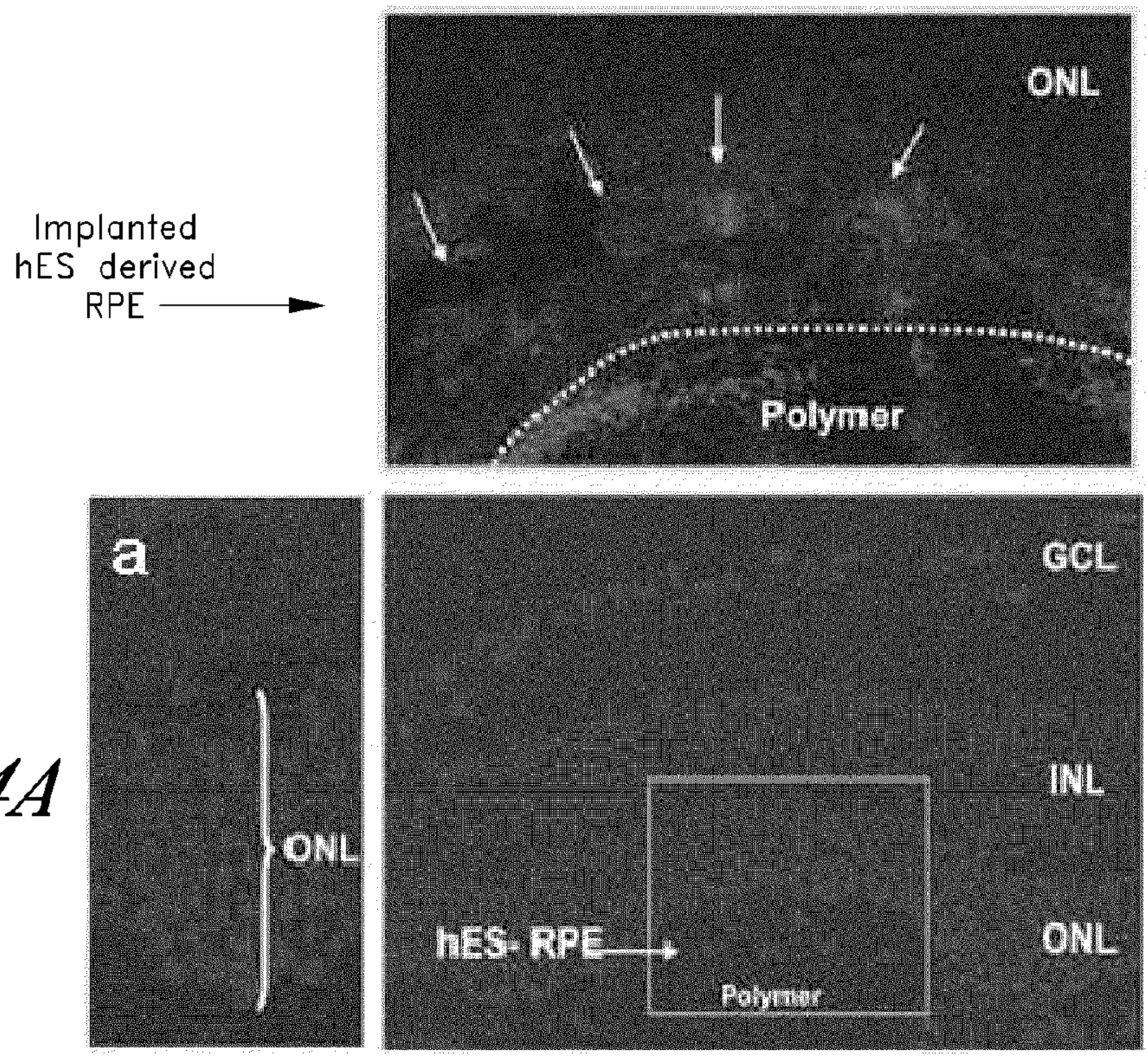



FIG. 14A

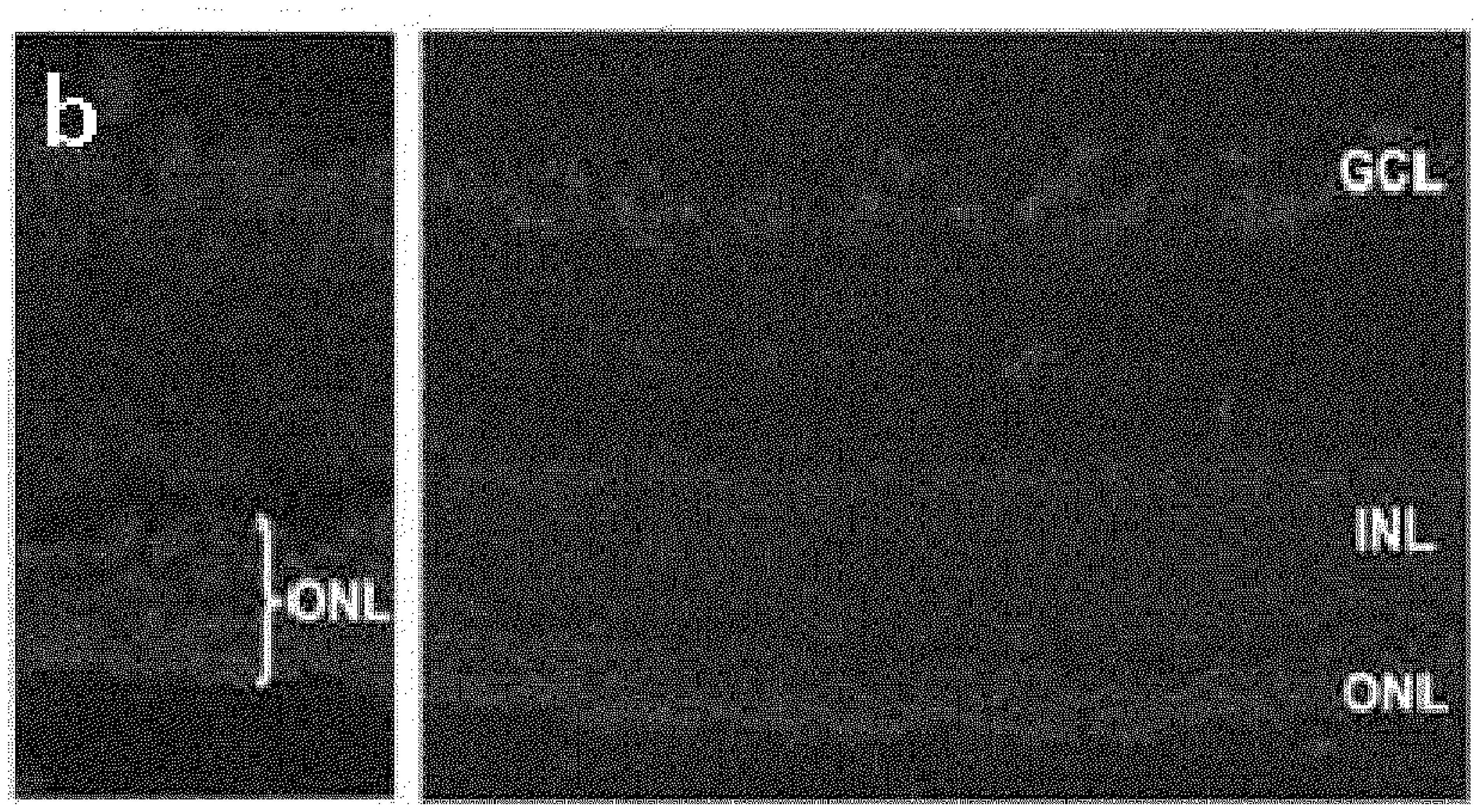



FIG. 14B

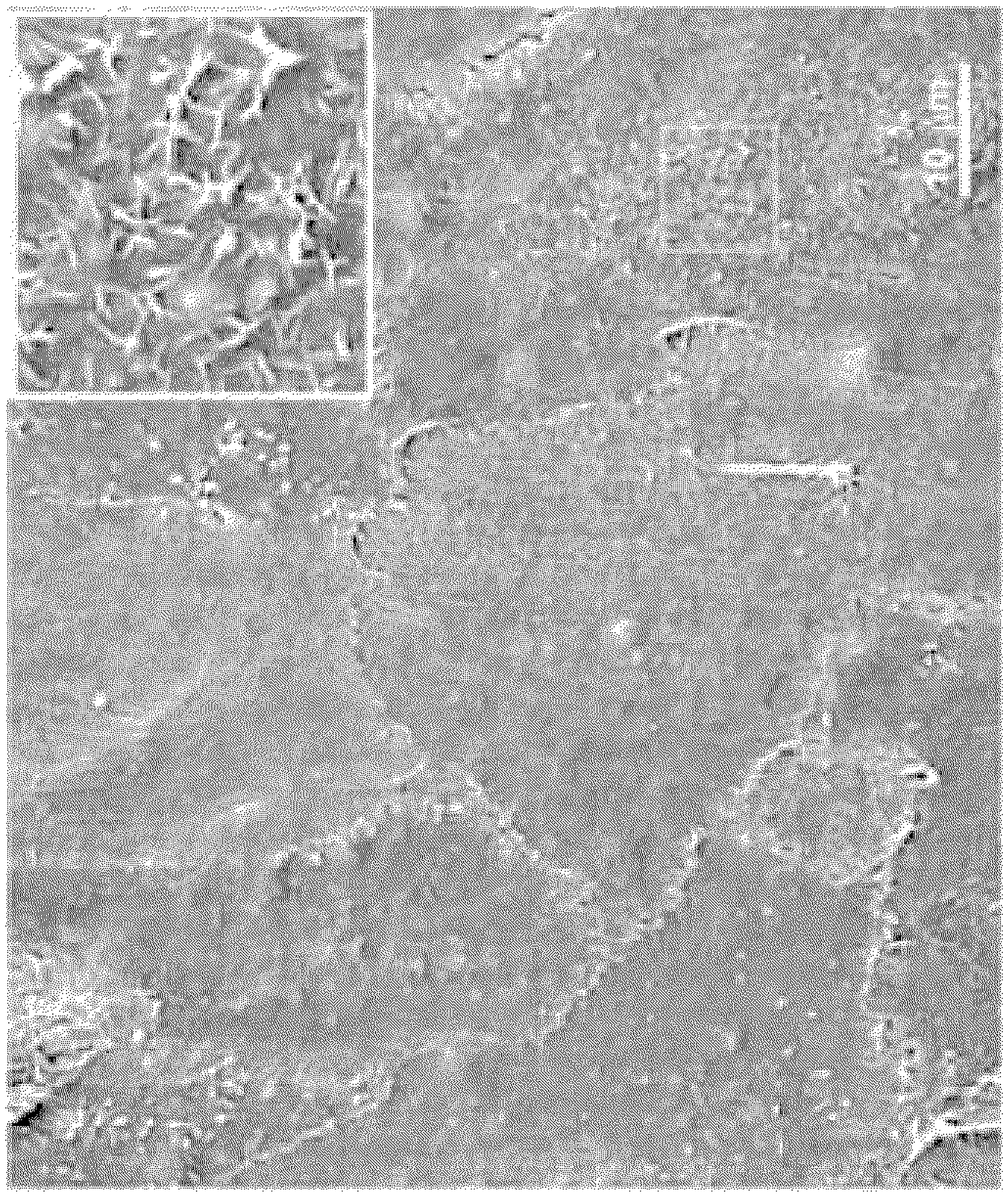


FIG. 15B

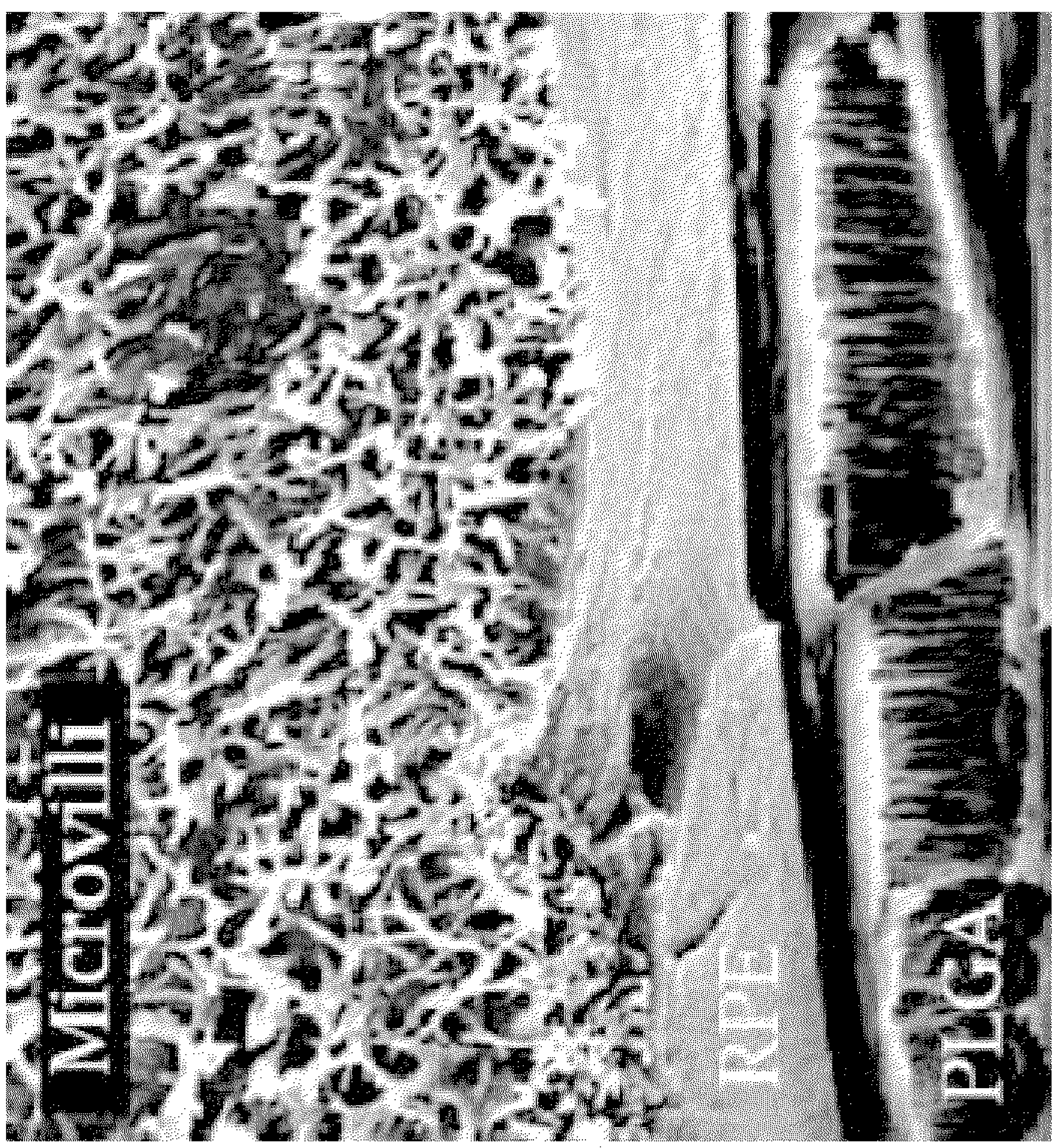



FIG. 15A

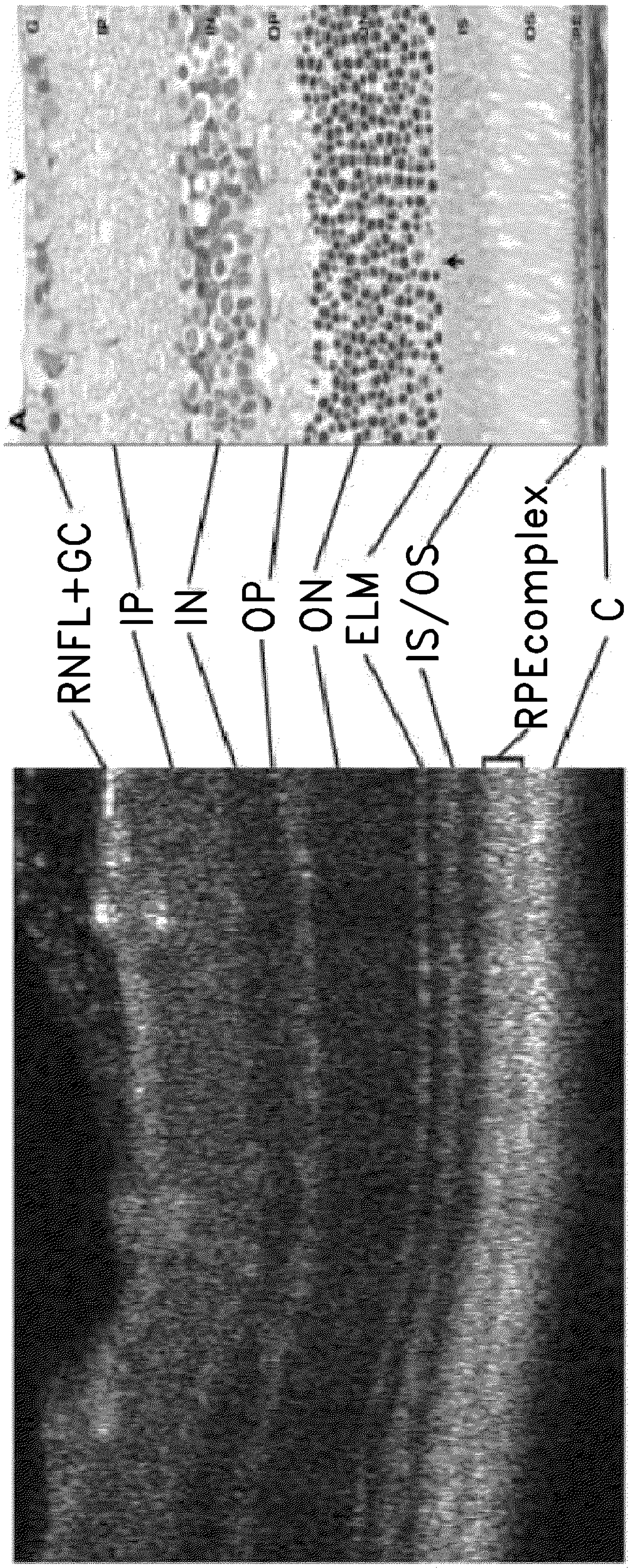



FIG. 16B

FIG. 16A

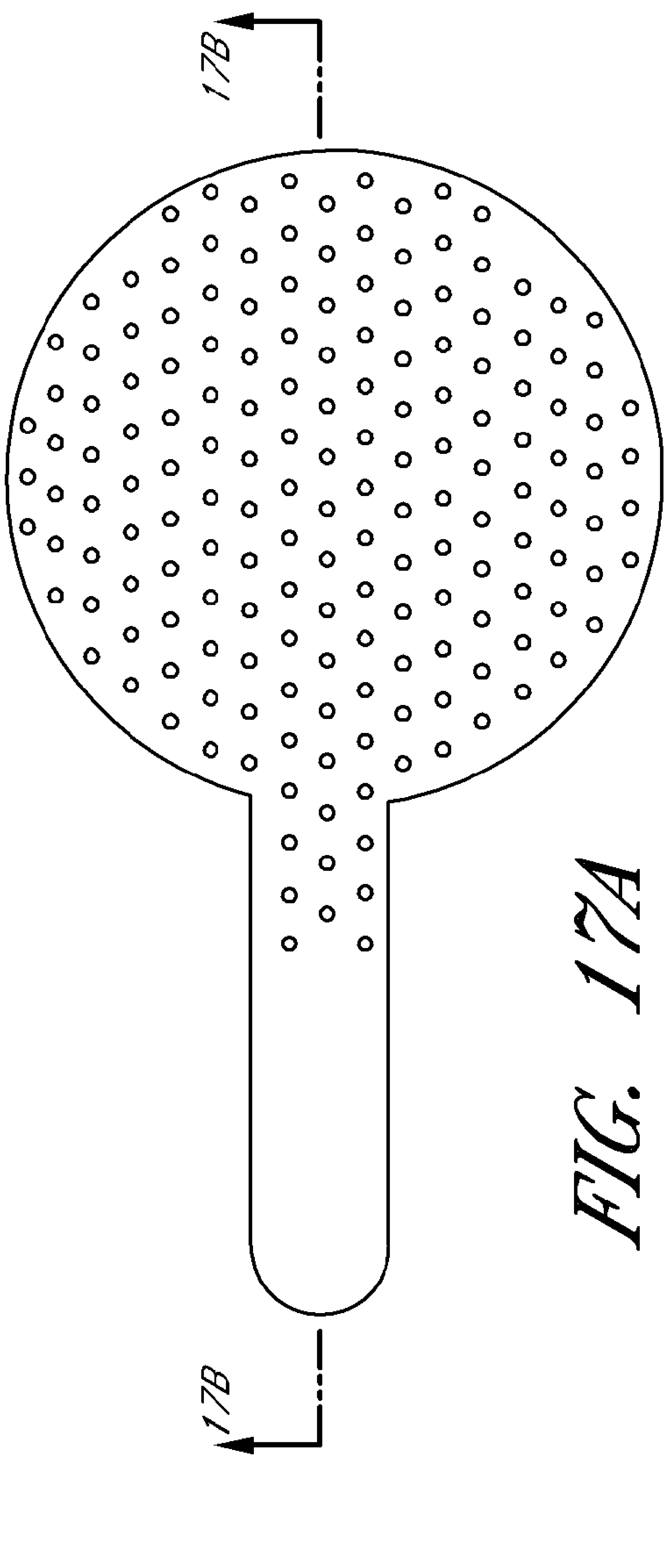



FIG. 17A

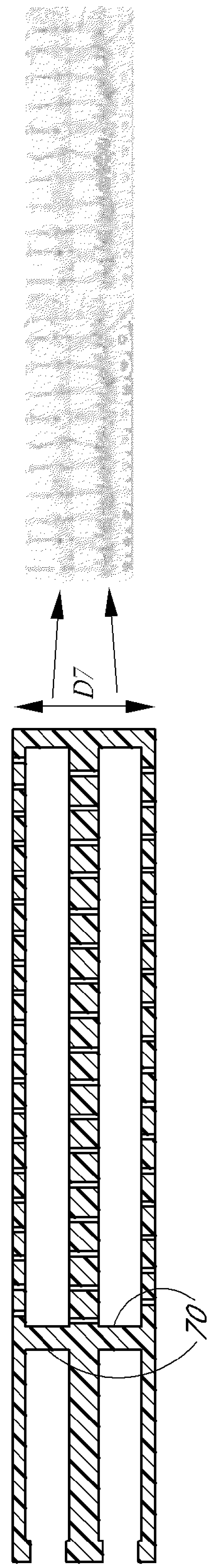



FIG. 17B

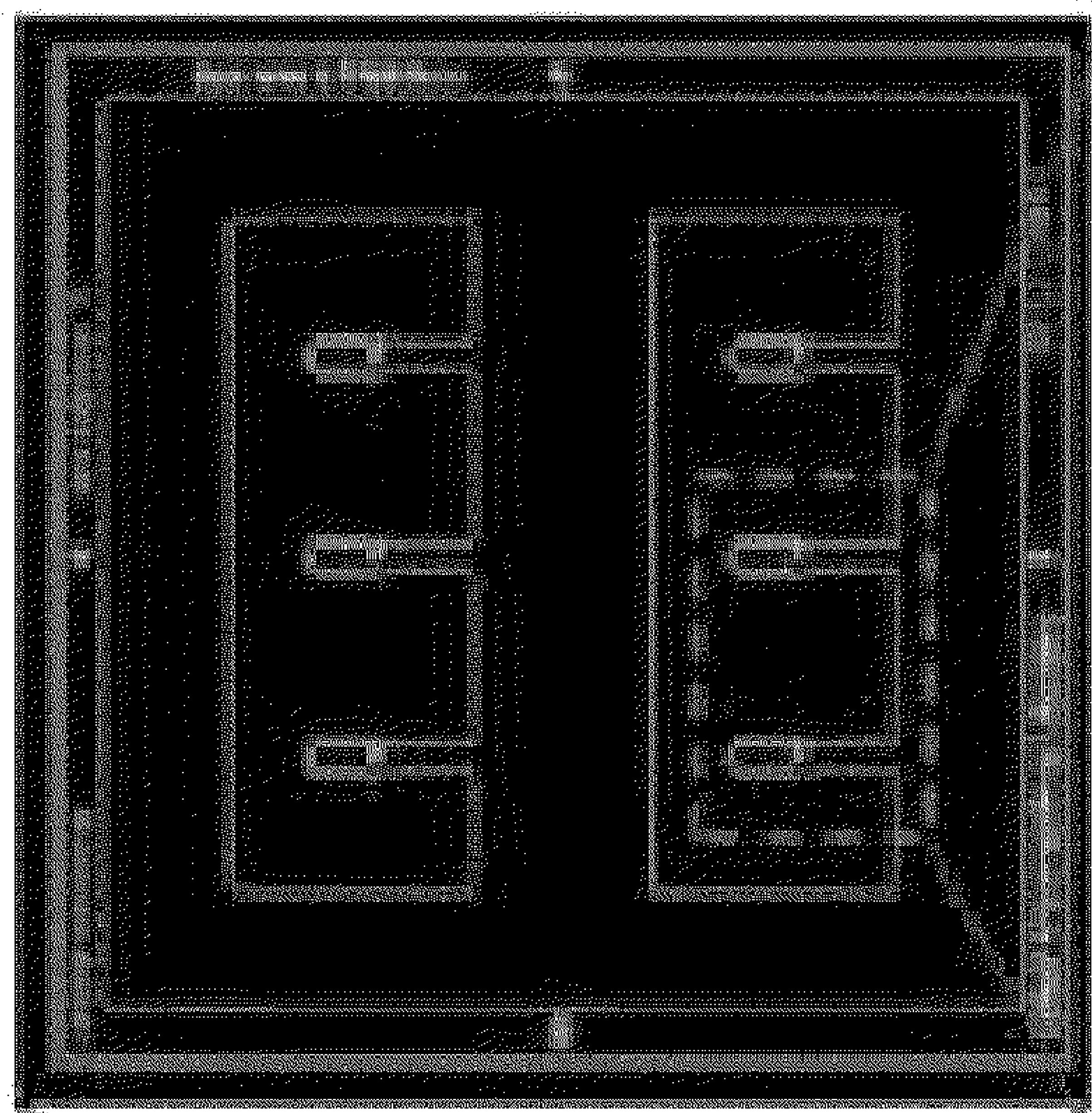


FIG. 18B

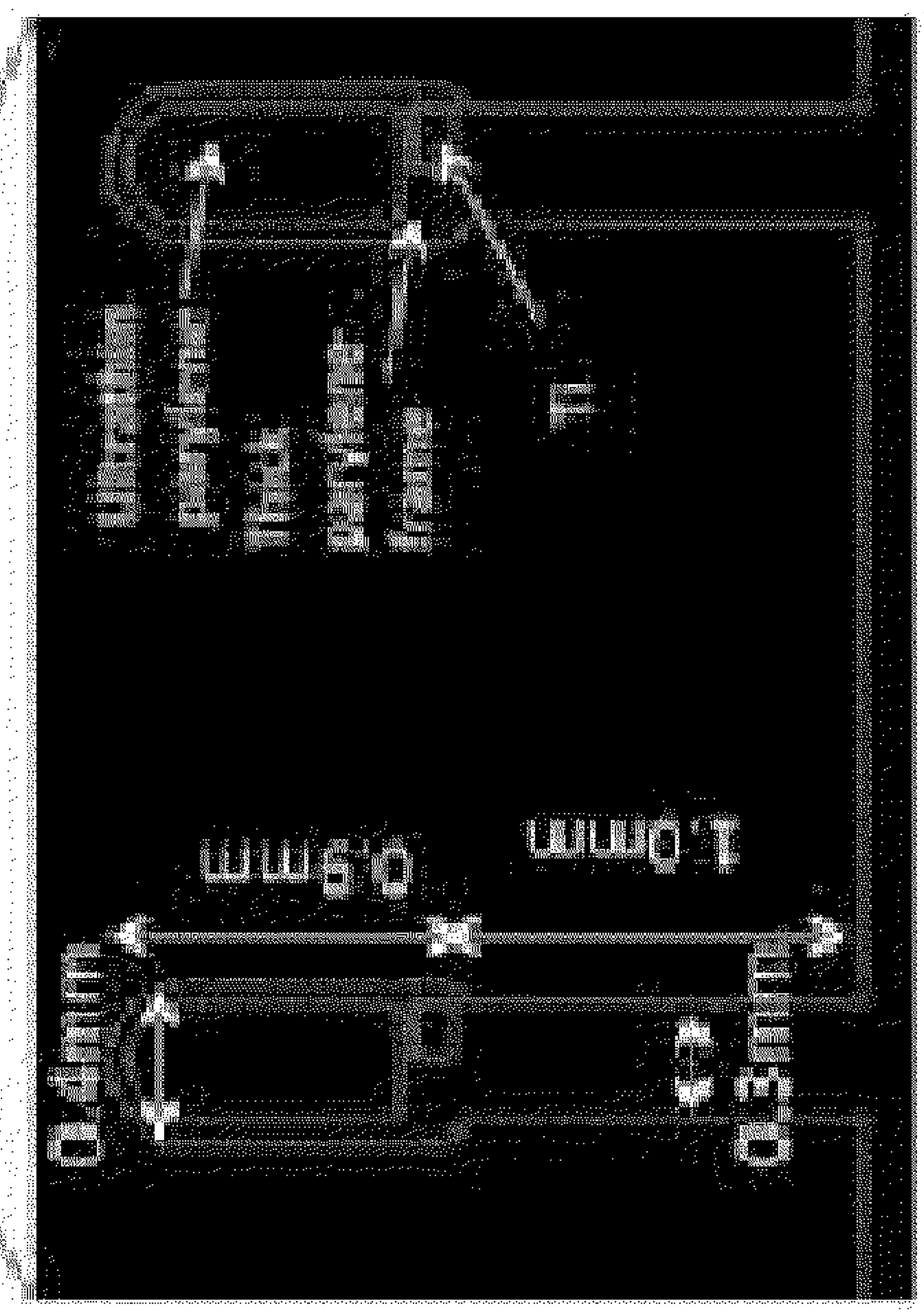



FIG. 18C

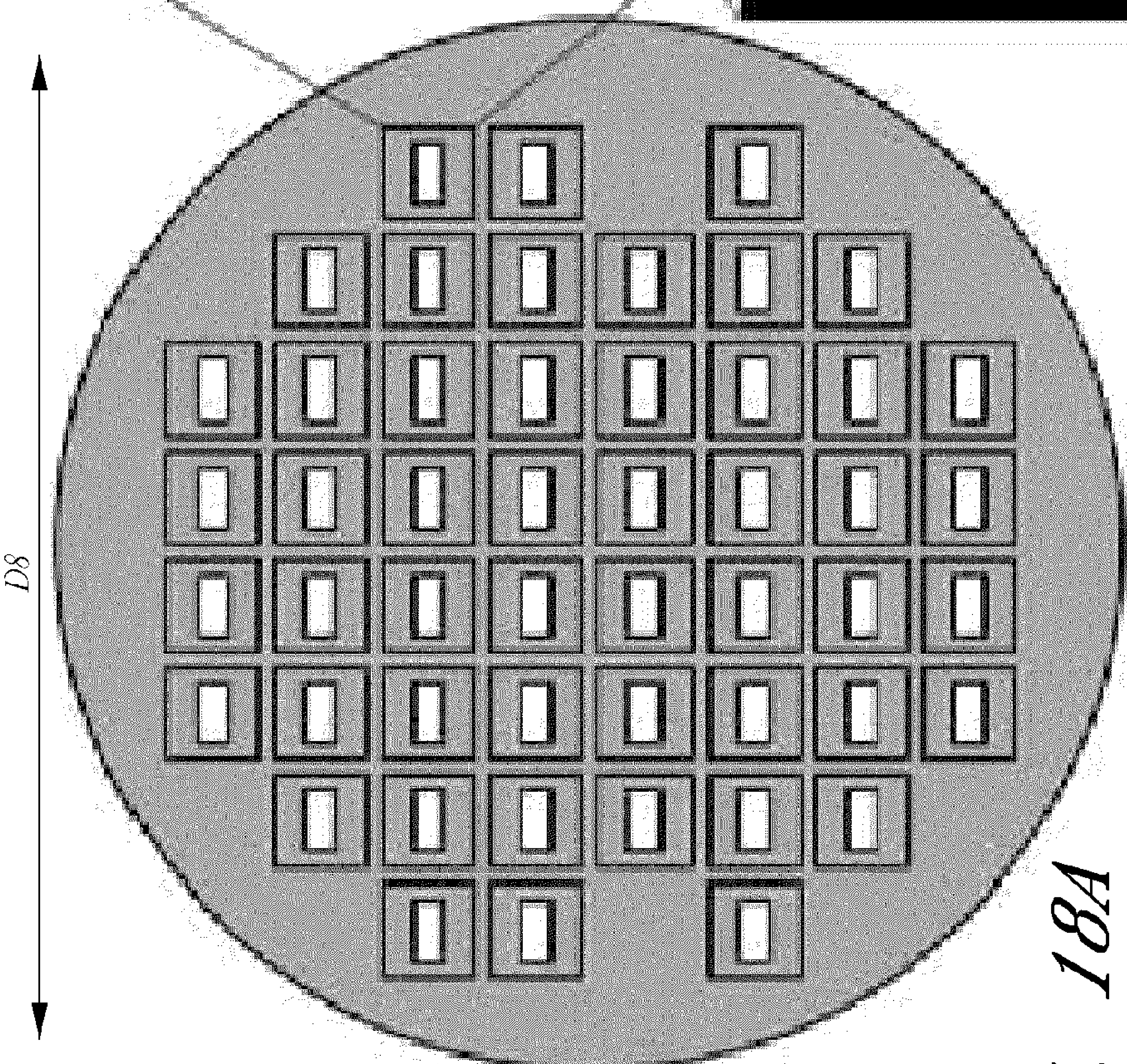



FIG. 18A

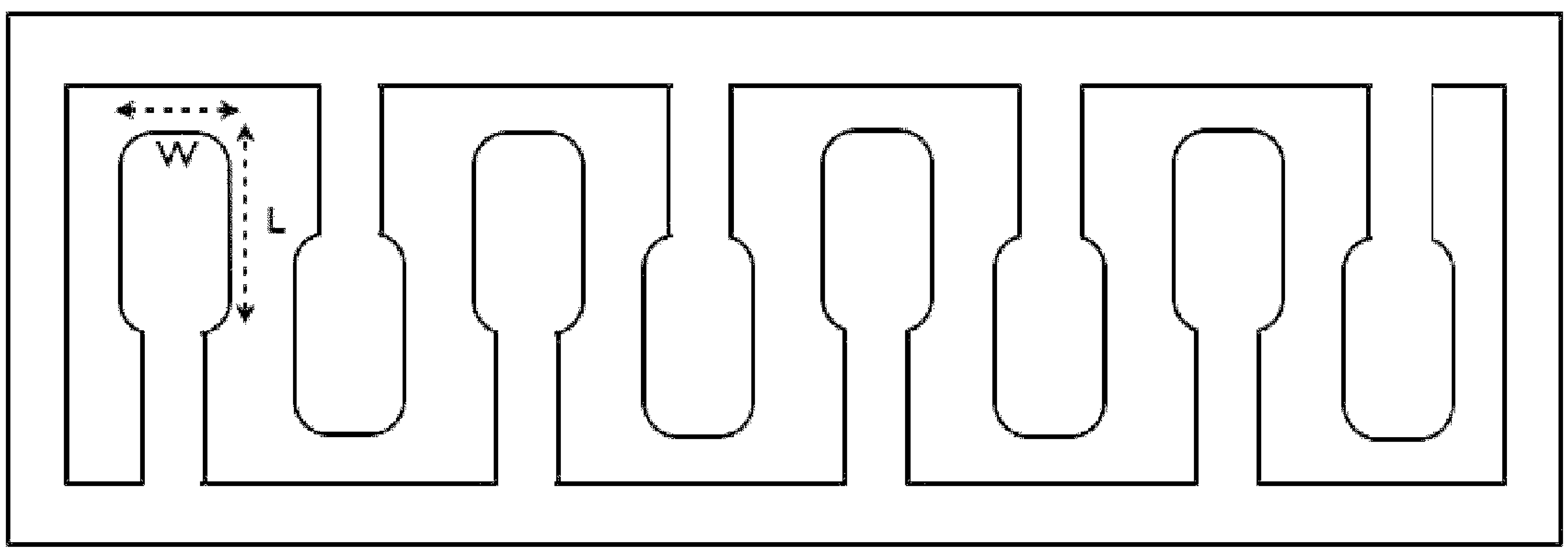



*FIG. 18D*

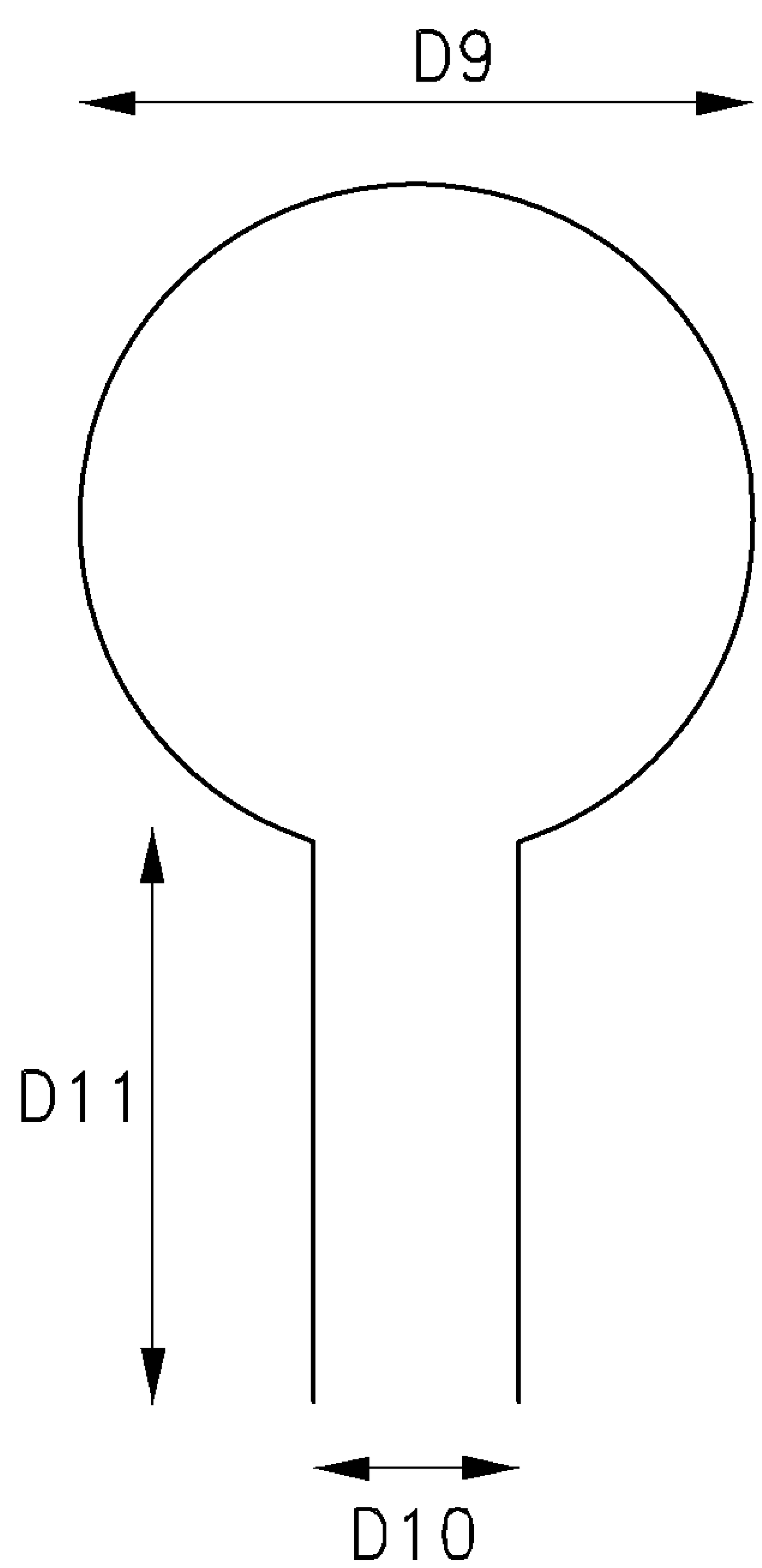



*FIG. 19A*

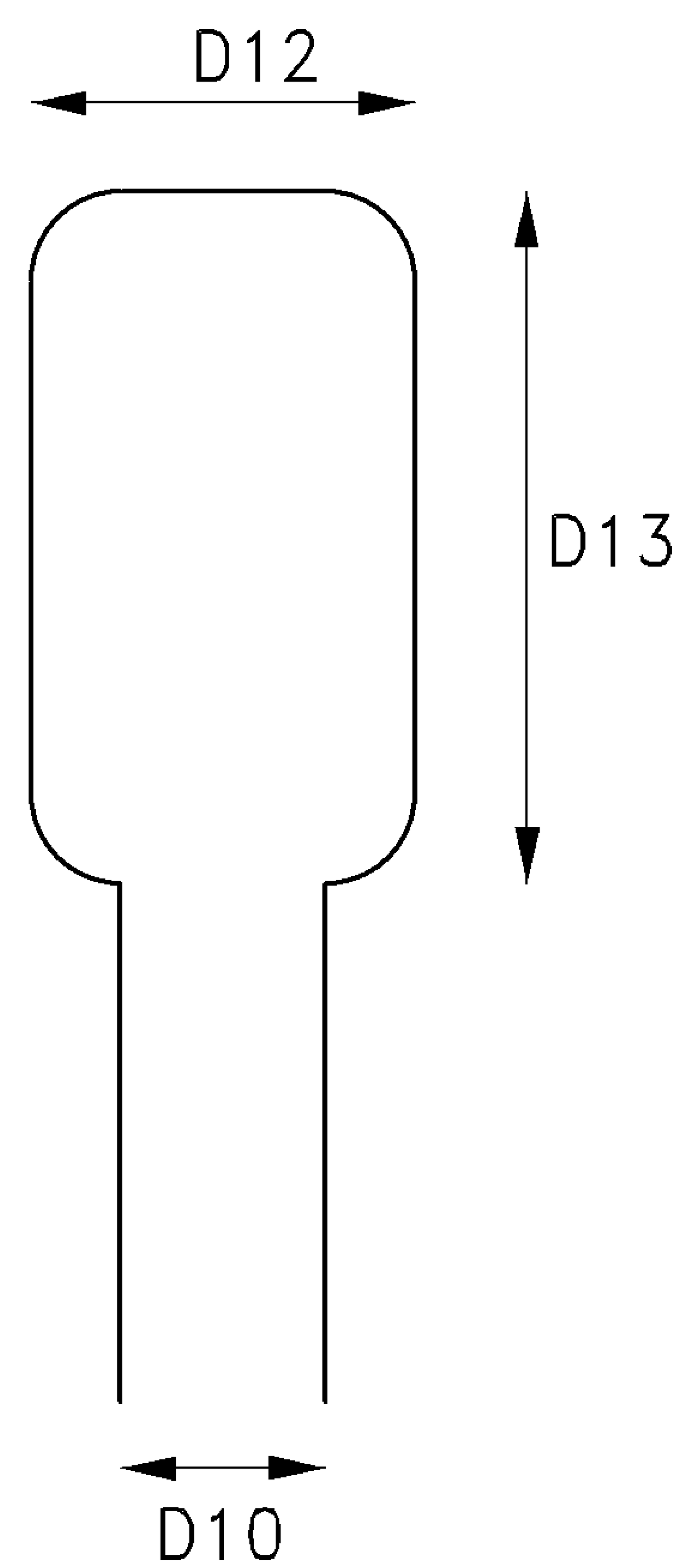



*FIG. 19B*

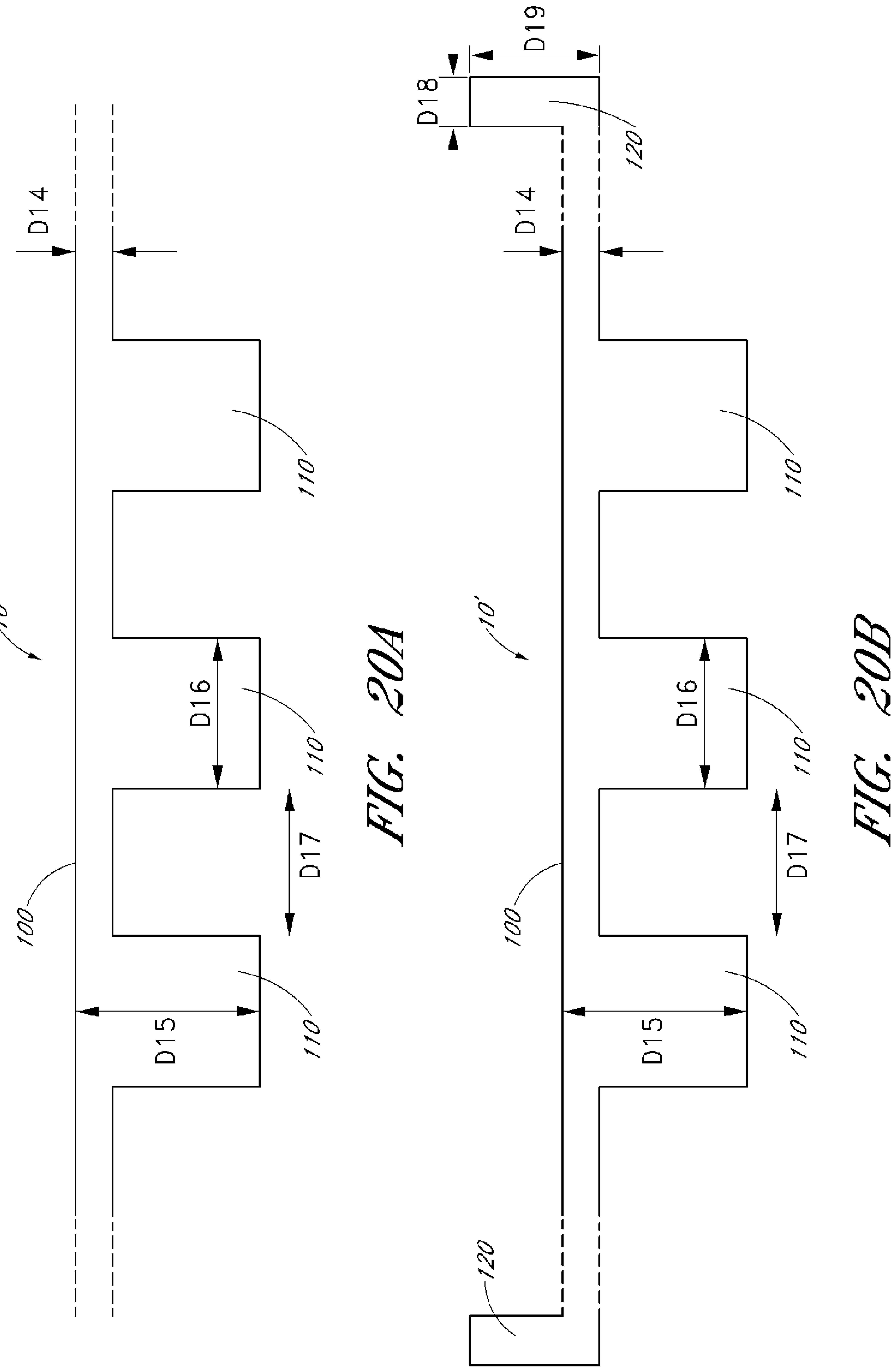



FIG. 20A

FIG. 20B

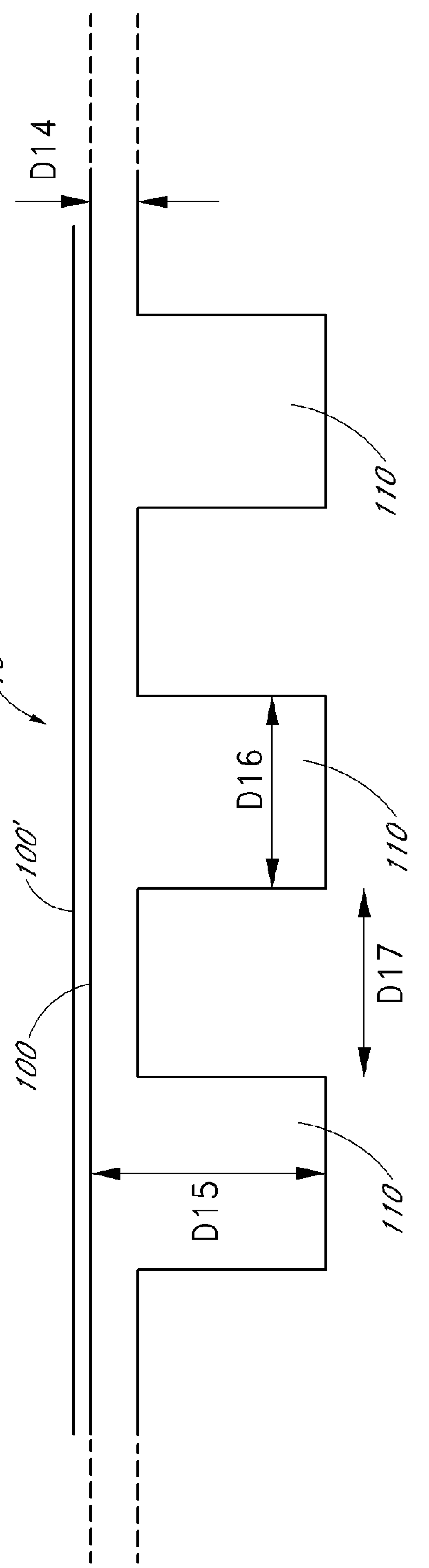



FIG. 20C

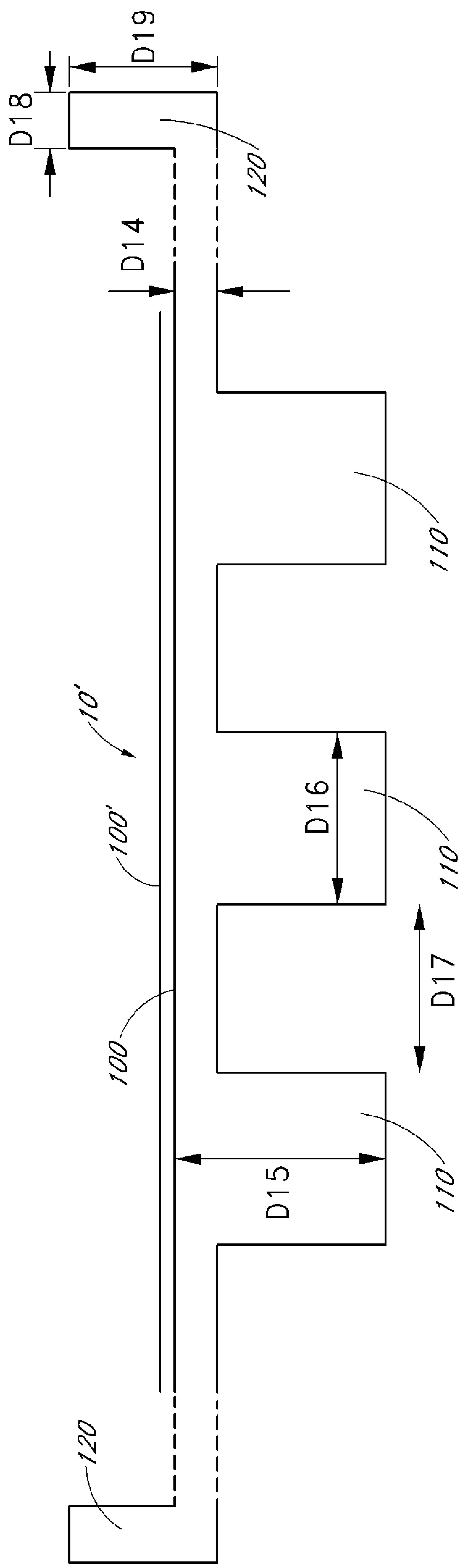



FIG. 20D

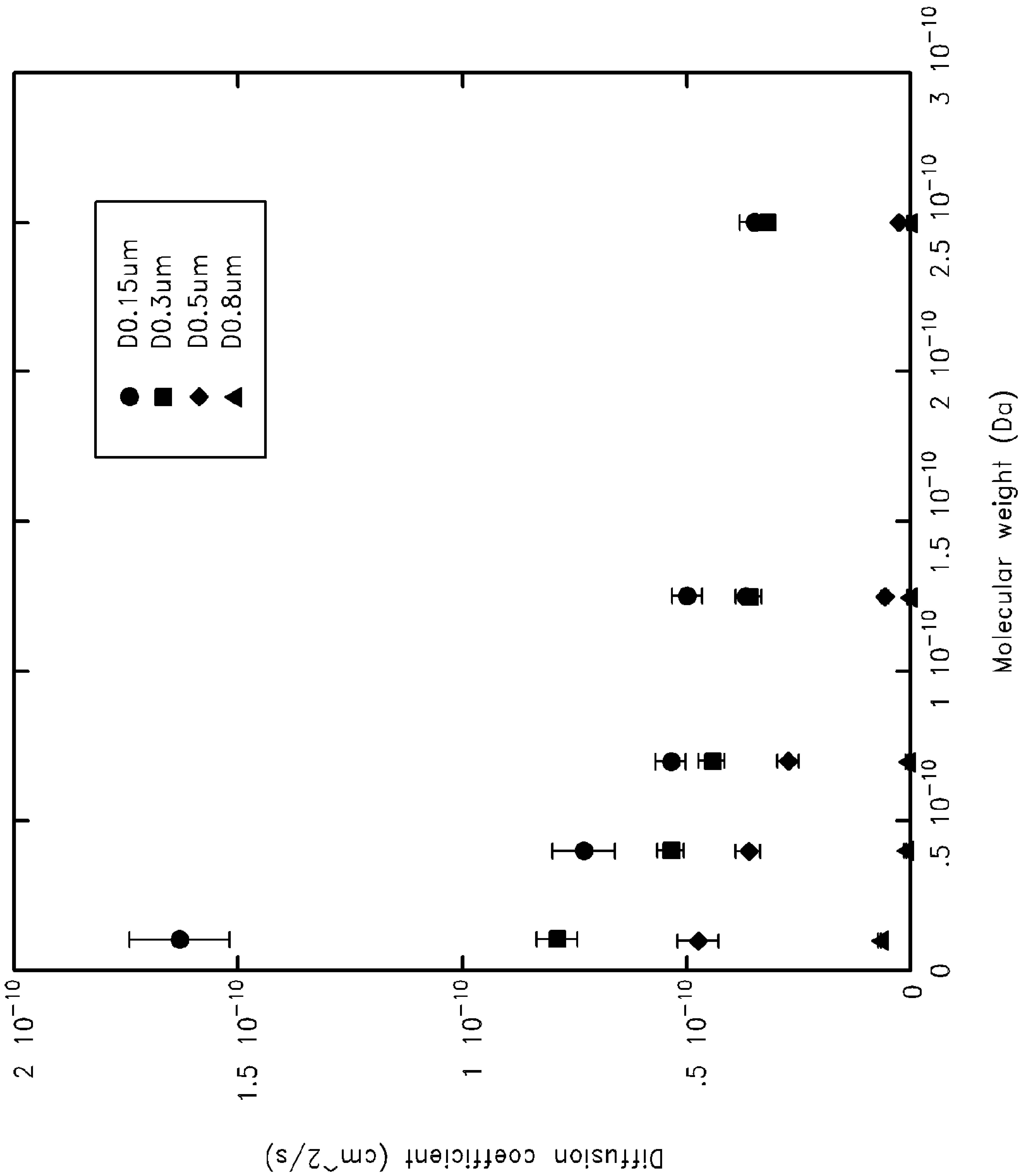

D0.15um
D0.3um
D0.5um
D0.8um
Molecular weight (Da)
Diffusion coefficient (cm^2/s)

*FIG. 20E*

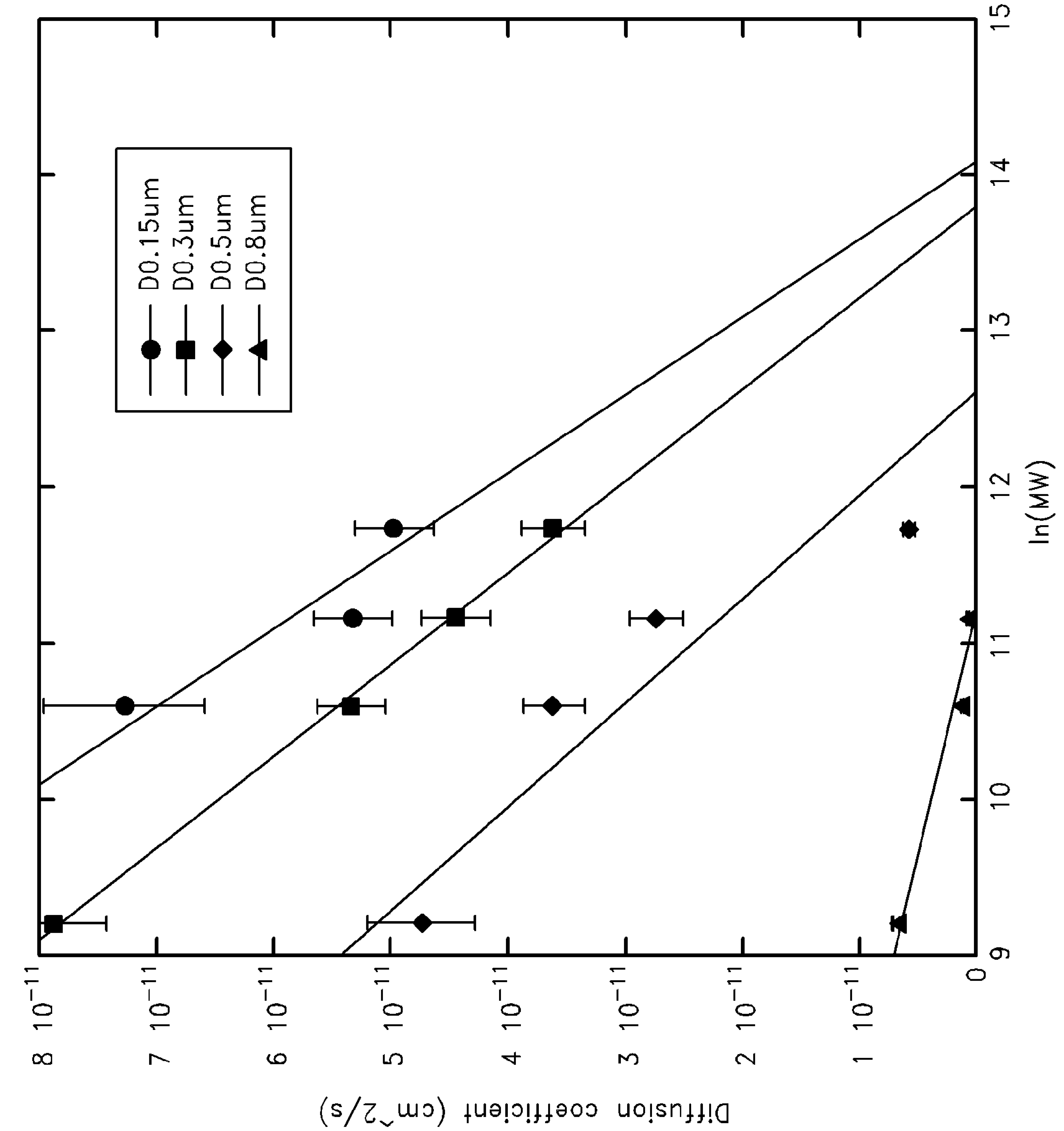

D0.15um
D0.3um
D0.5um
D0.8um
Diffusion coefficient (cm^2/s)
0
1 10^-11
2 10^-11
3 10^-11
4 10^-11
5 10^-11
6 10^-11
7 10^-11
8 10^-11
ln(MW)
9
10
11
12
13
14
15

*FIG. 20F*

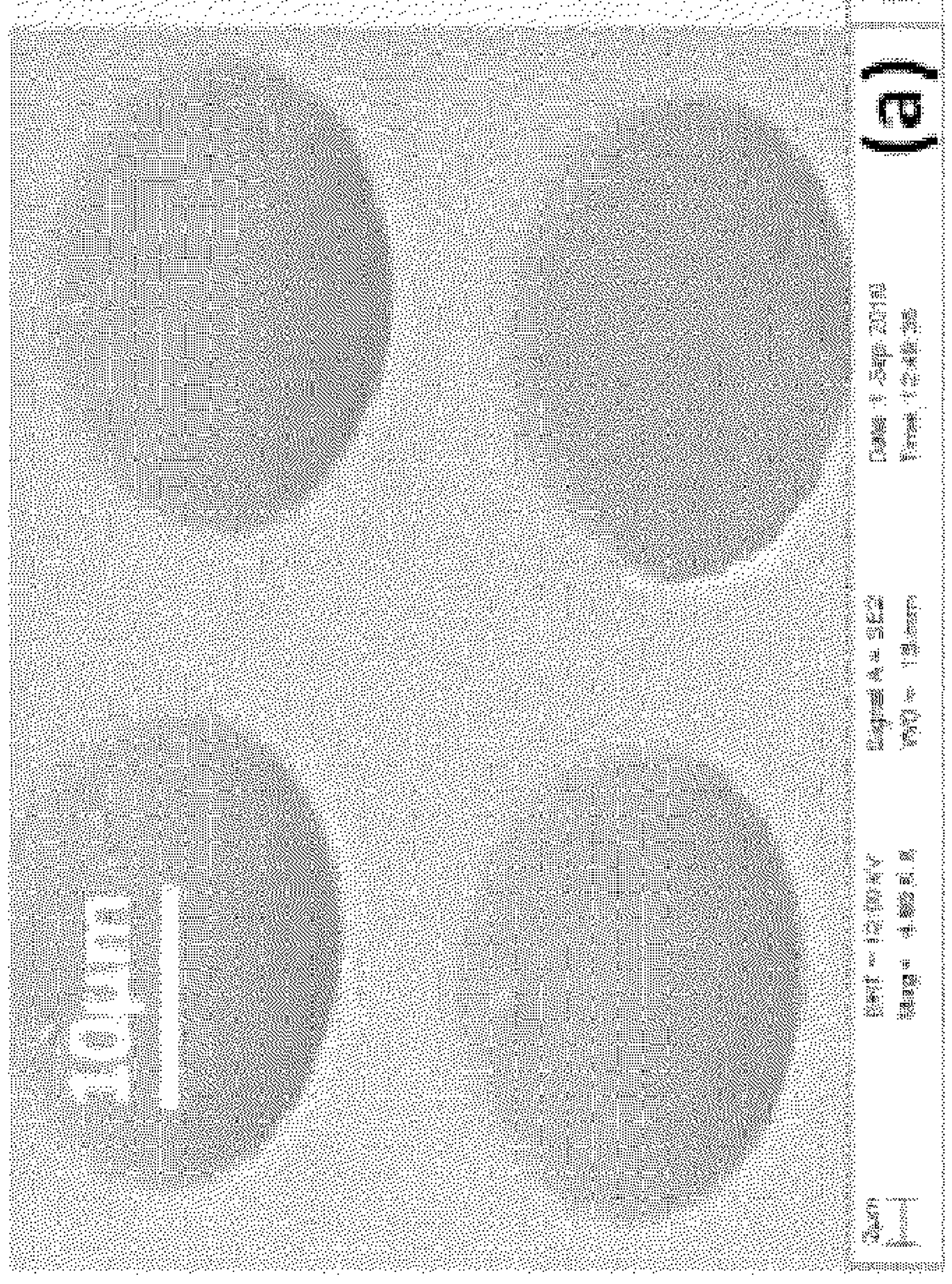



FIG. 21A


FIG. 21B

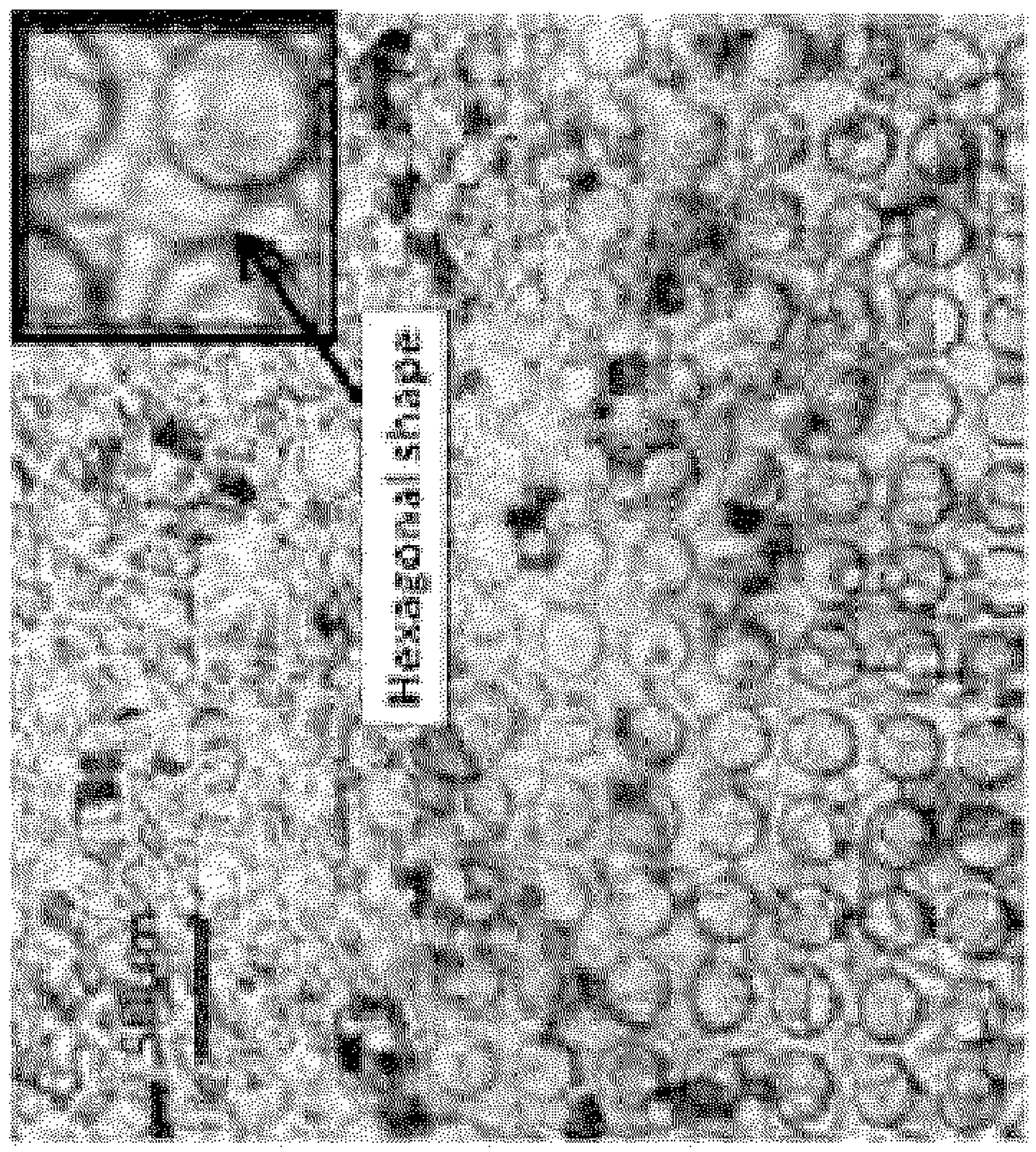



FIG. 21C

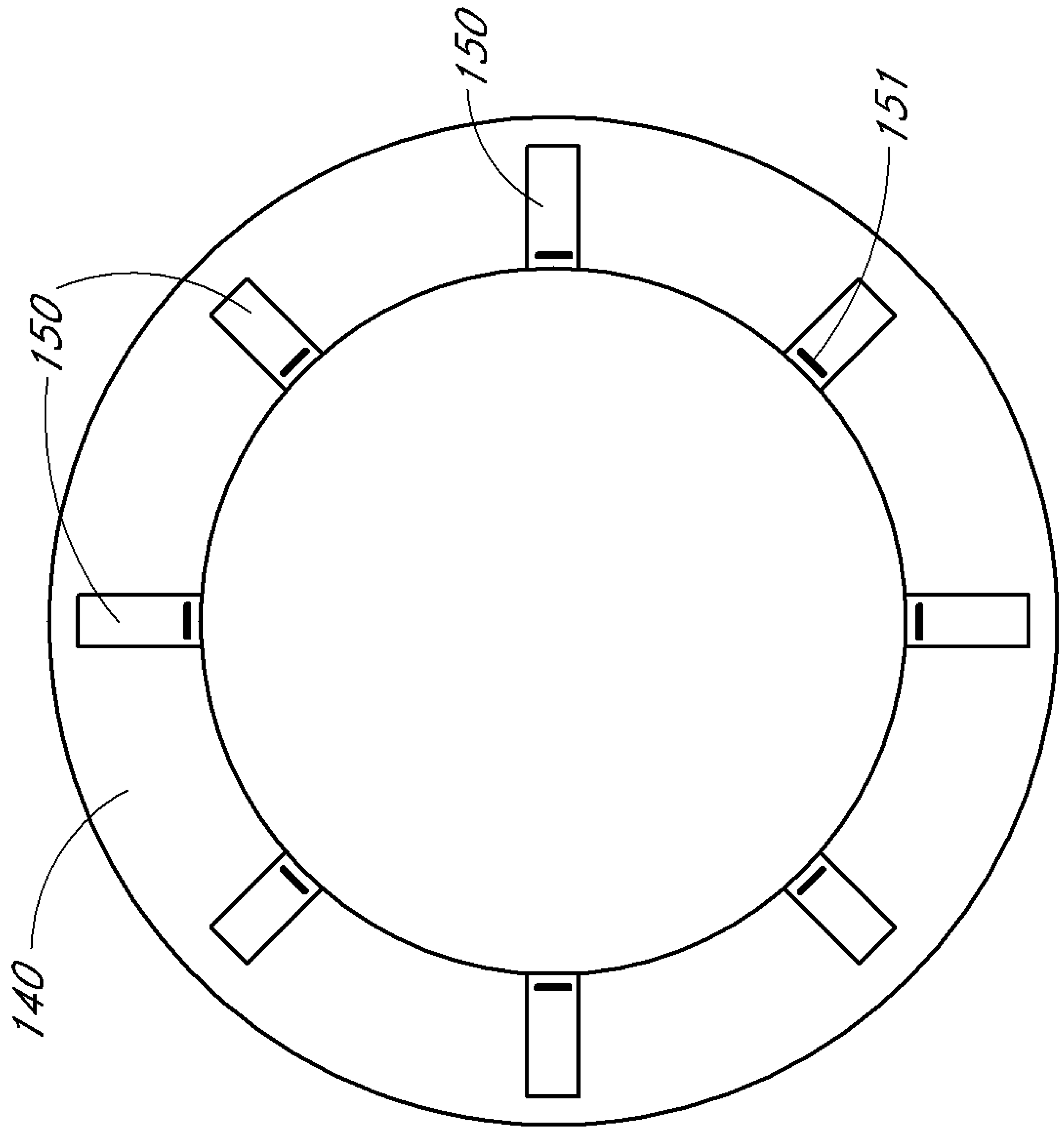



FIG. 22B

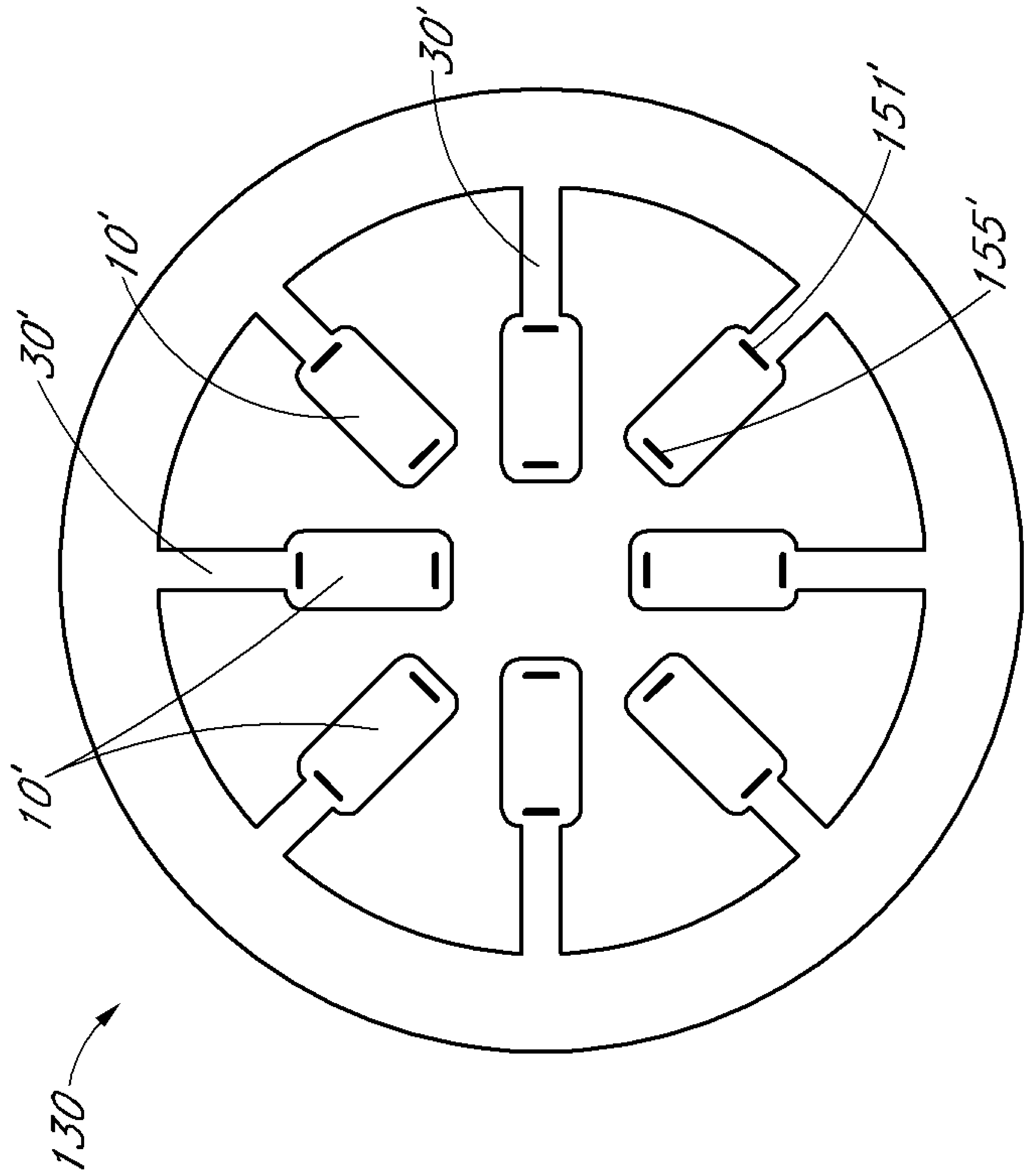



FIG. 22A

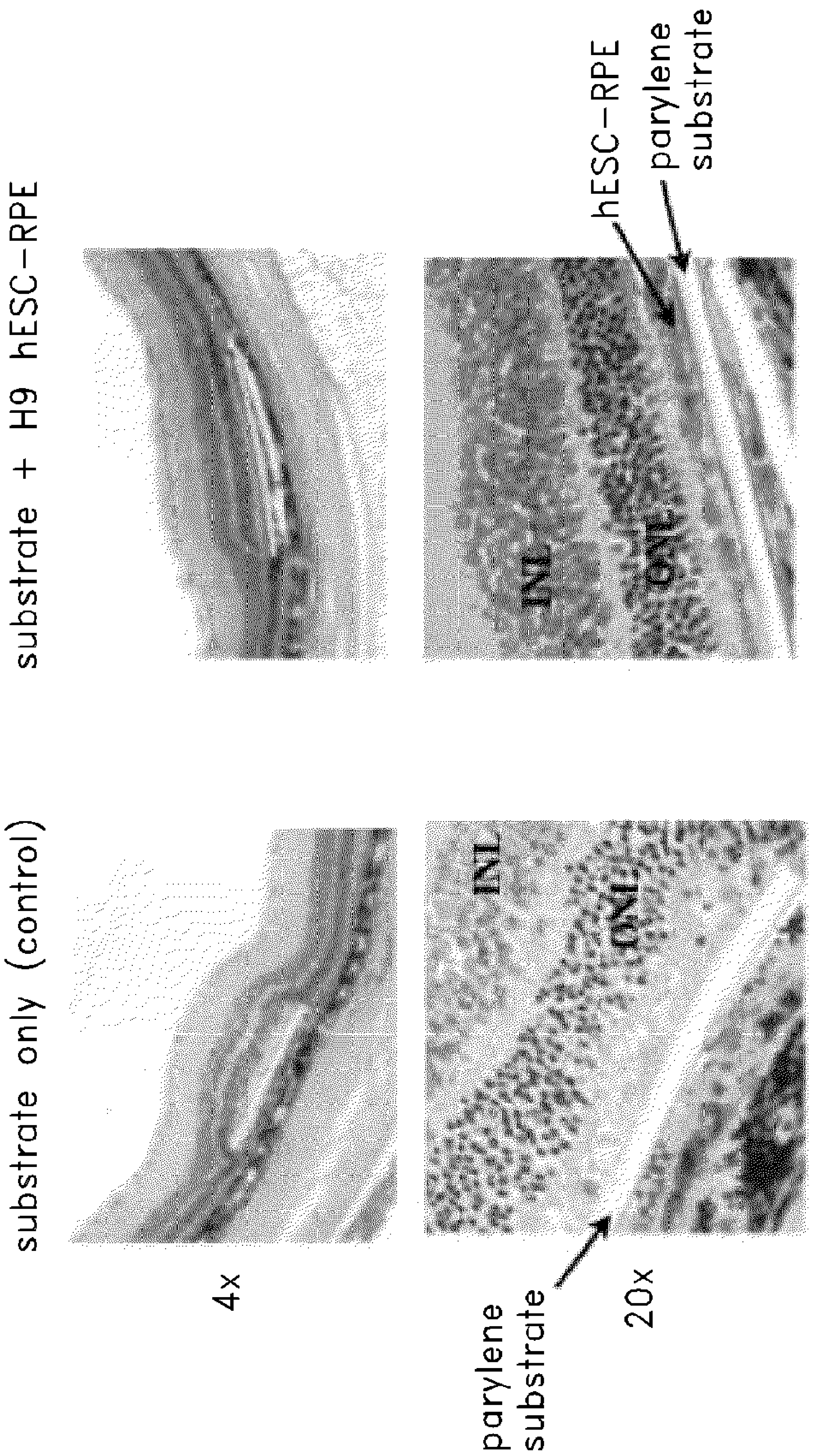



FIG. 23B

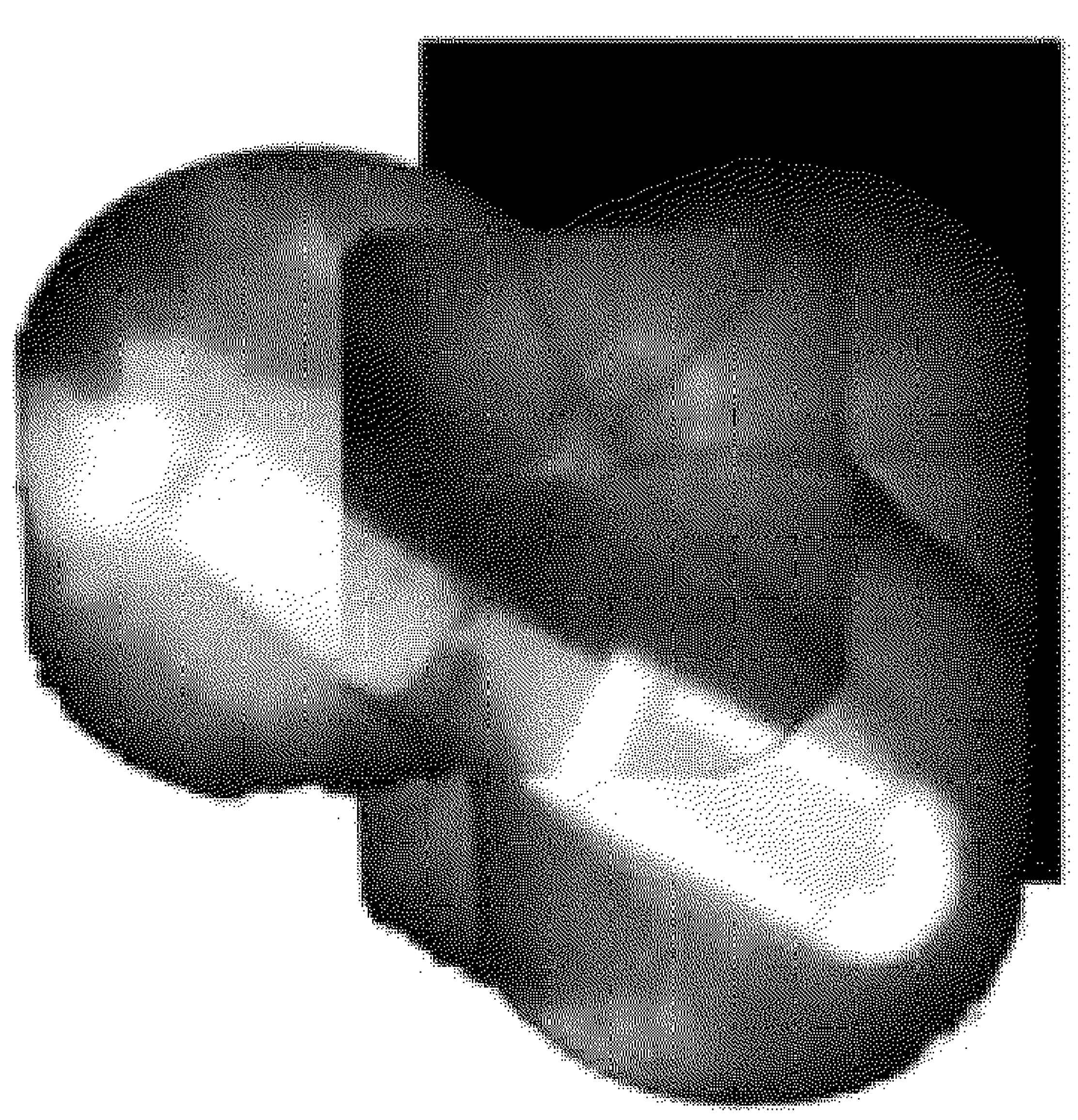
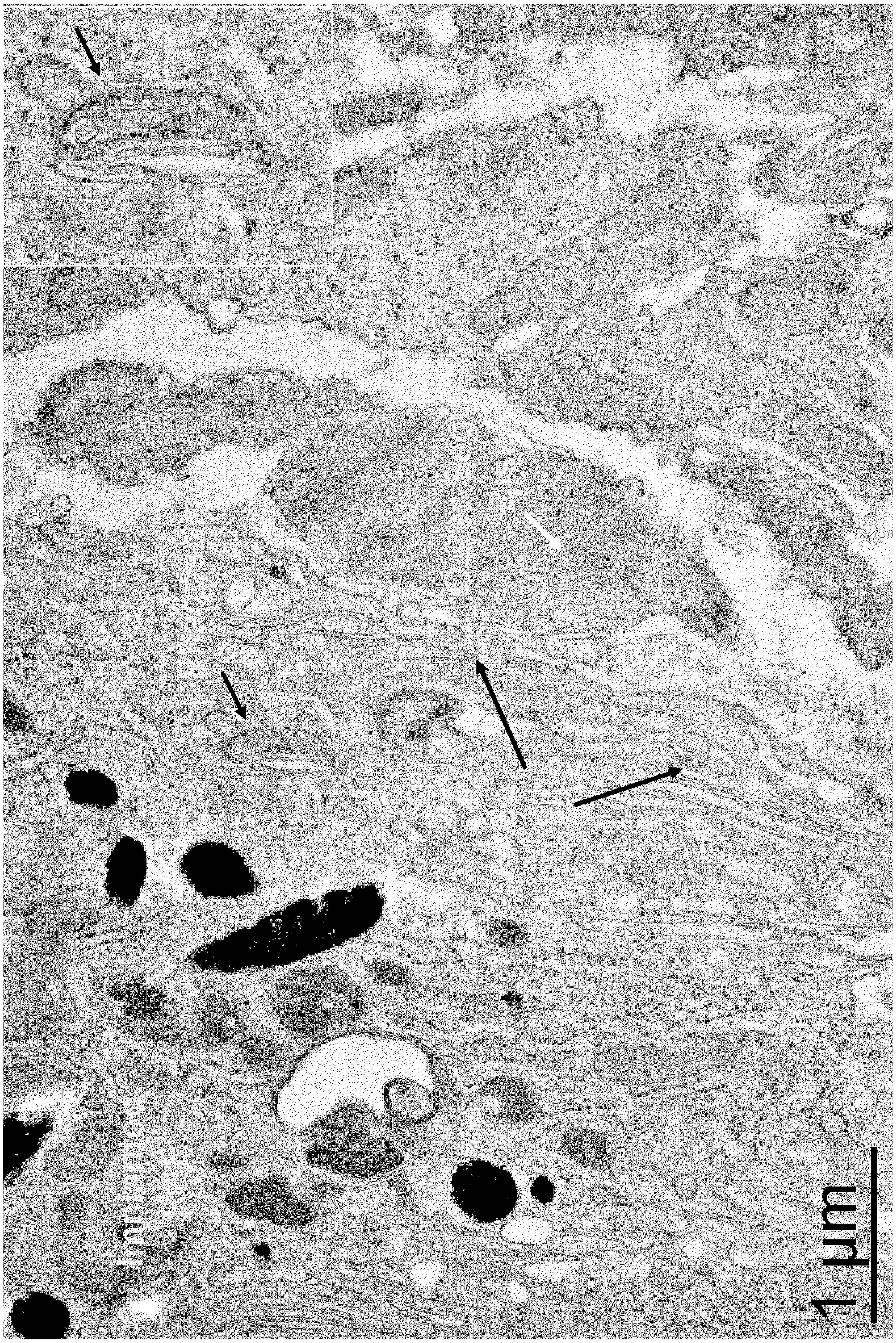


FIG. 23A


FIG. 23D

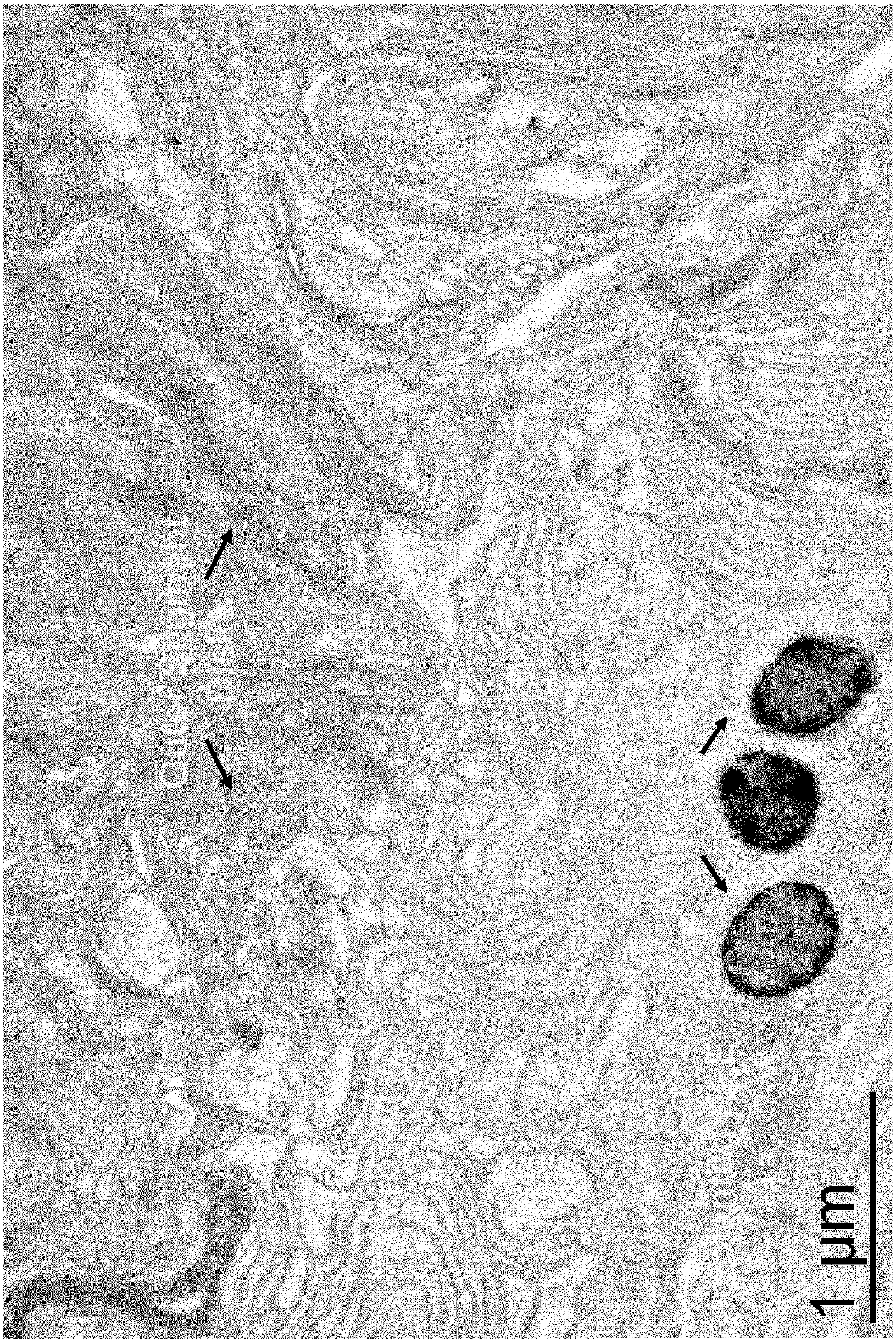
FIG. 23C

*FIG. 24*




Post Implant Day 9

*FIG. 25*




Post Implant Day 58

FIG. 26

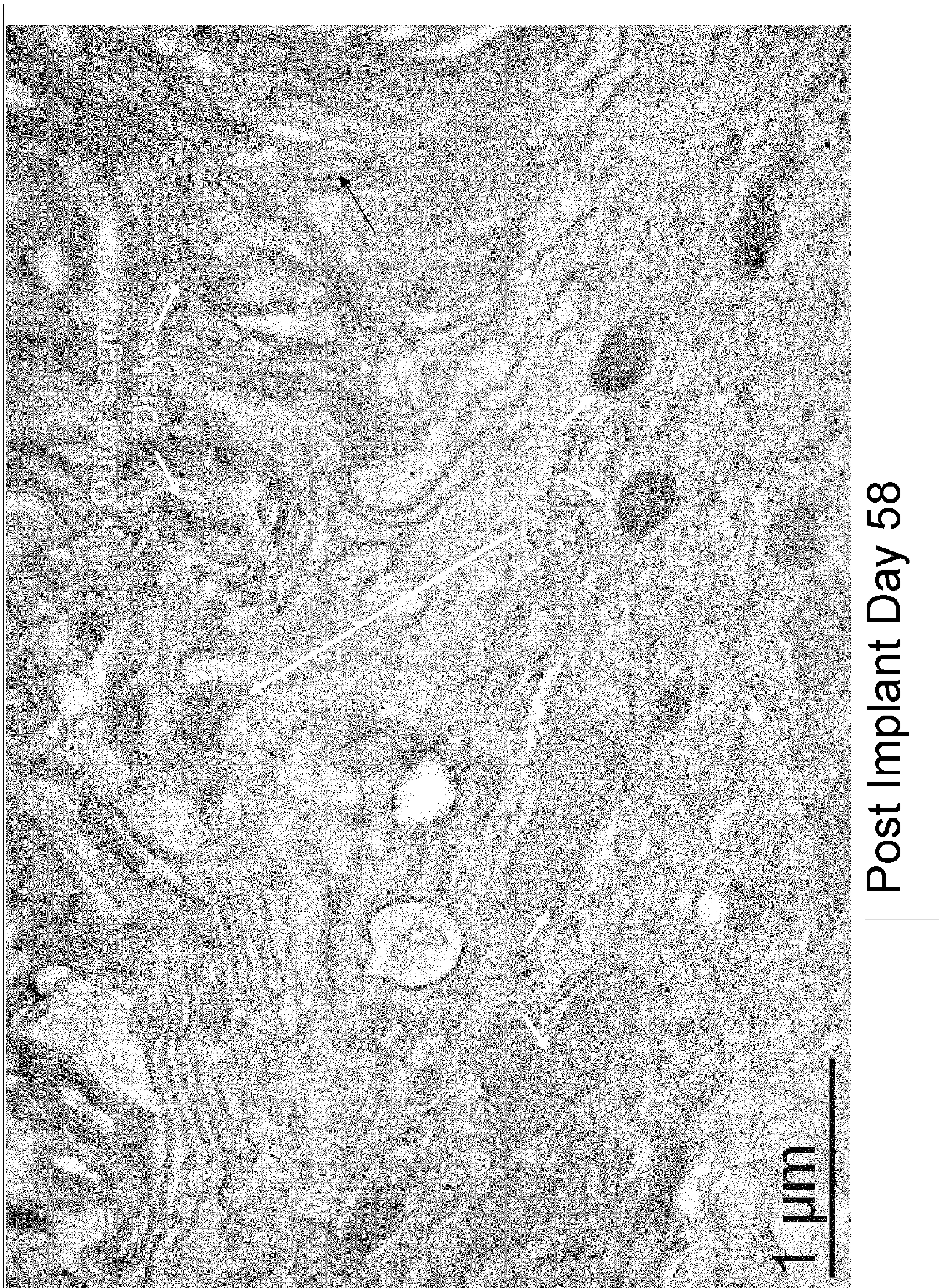
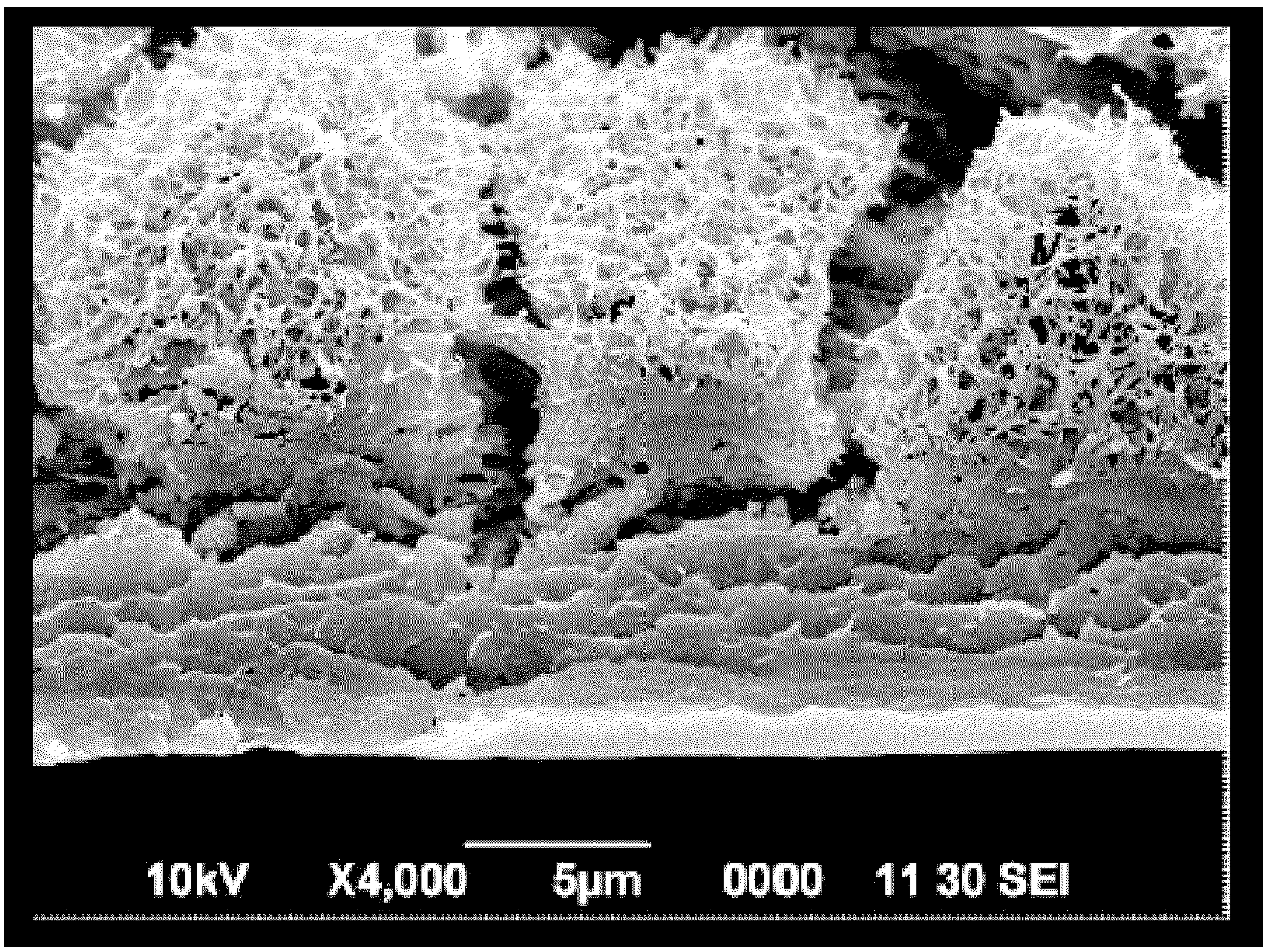
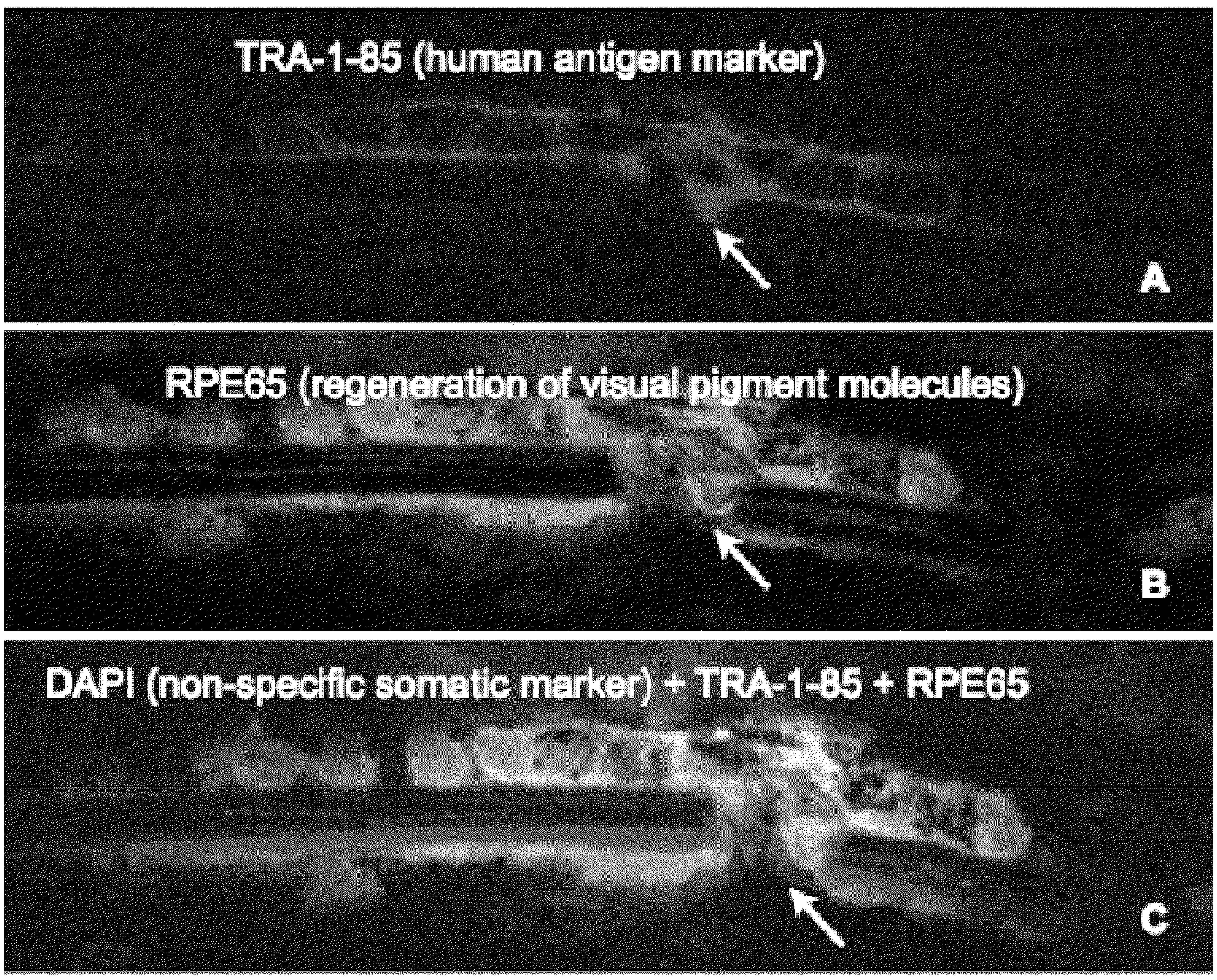


FIG. 27



*FIGS. 28A-28C*


TRA-1-85 (human antigen marker)
A
RPE65 (regeneration of visual pigment molecules)
B
DAPI (non-specific somatic marker) + TRA-1-85 + RPE65
C

*FIGS. 29A-29C*

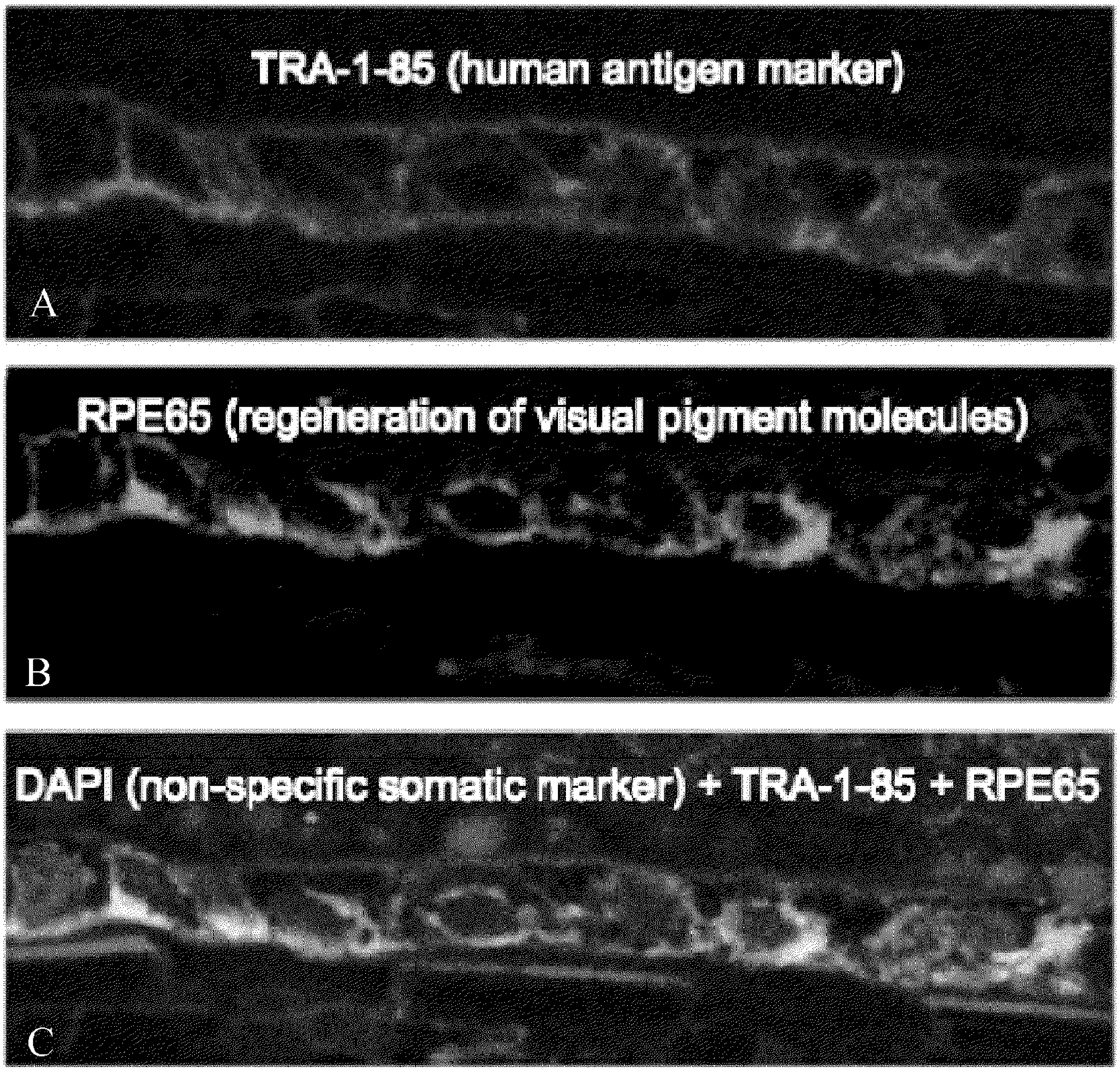

TRA-1-85 (human antigen marker)
A
RPE65 (regeneration of visual pigment molecules)
B
DAPI (non-specific somatic marker) + TRA-1-85 + RPE65
C

*FIG. 30*

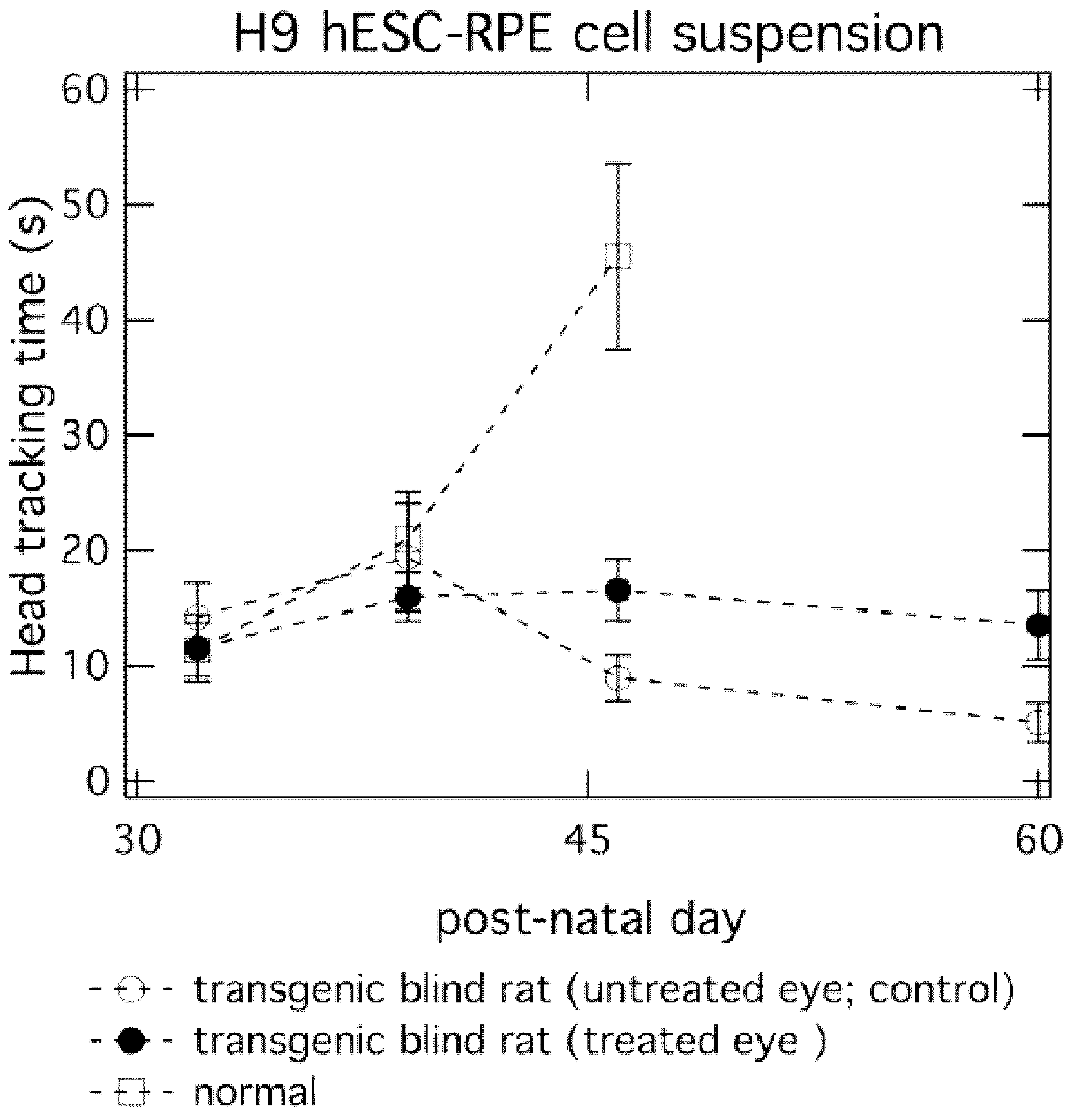

H9 hESC-RPE cell suspension
Head tracking time (s)
0
10
20
30
40
50
60
30
45
60
post-natal day
transgenic blind rat (untreated eye; control)
transgenic blind rat (treated eye )
normal

FIG. 31

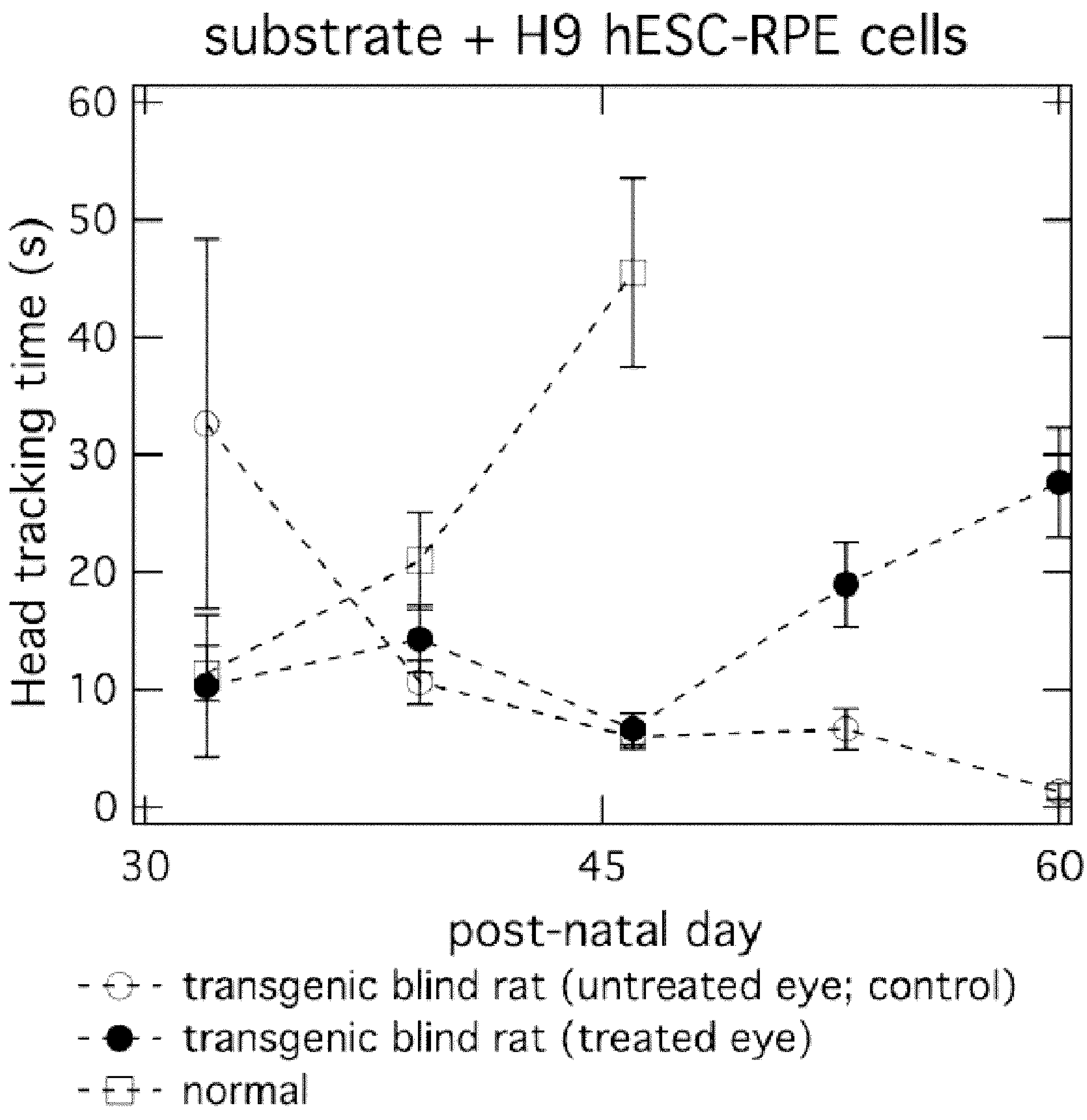
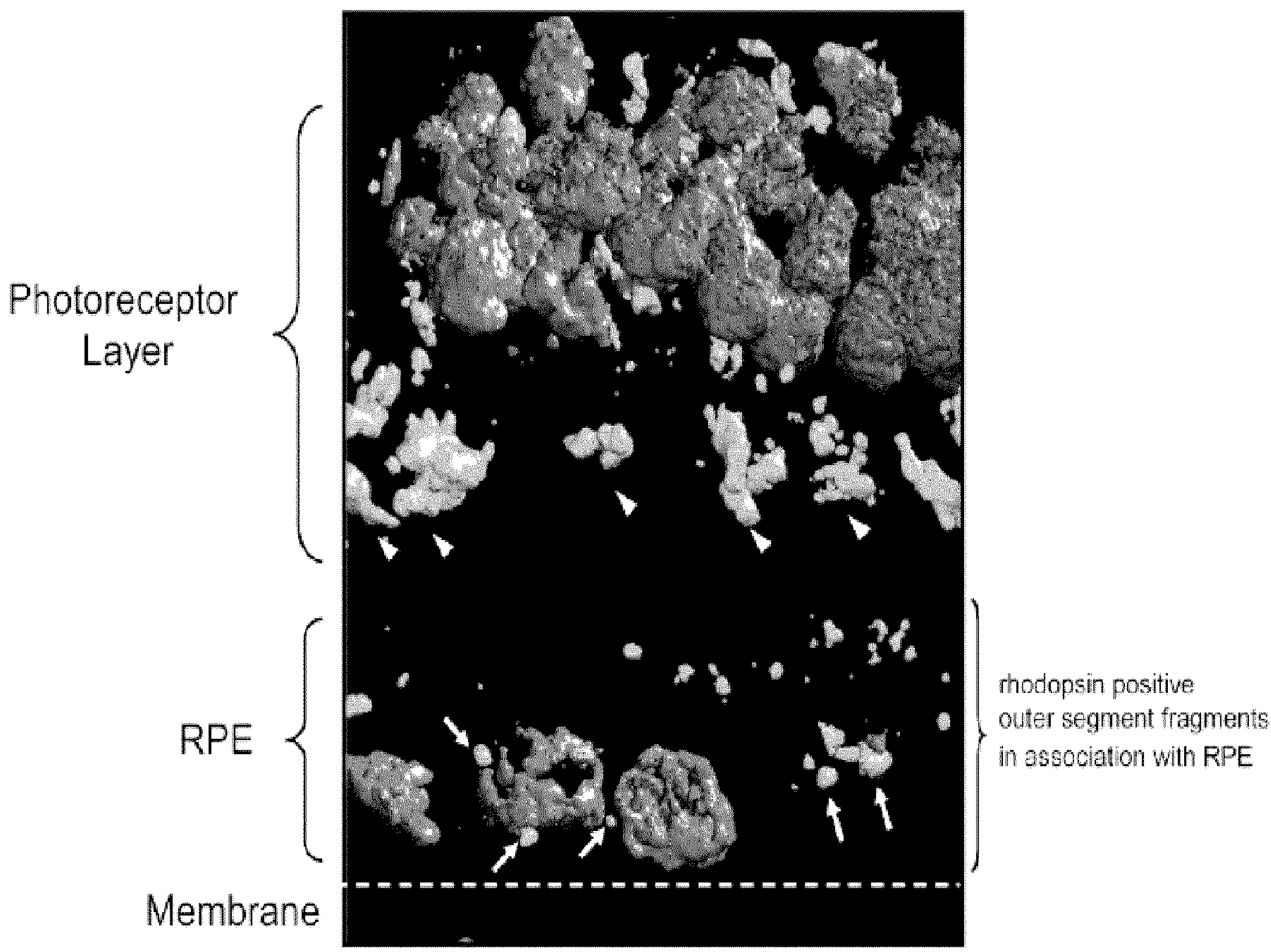

substrate + H9 hESC-RPE cells
Head tracking time (s)
0
10
20
30
40
50
60
30
45
60
post-natal day
transgenic blind rat (untreated eye; control)
transgenic blind rat (treated eye)
normal

*FIG. 32*


Photoreceptor Layer
RPE
Membrane
rhodopsin positive
outer segment fragments
in association with RPE

# BIOCOMPATIBLE SUBSTRATE FOR FACILITATING INTERCONNECTIONS BETWEEN STEM CELLS AND TARGET TISSUES AND METHODS FOR IMPLANTING SAME

## RELATED CASES

This application claims the benefit of U.S. Provisional Application Ser. Nos. 61/363,630, filed on Jul. 12, 2010 and 61/481,004, filed on Apr. 29, 2011, the contents of each of which is expressly incorporated in its entirety by reference herein.

## BACKGROUND

1. Field of the Invention

The present application relates generally to substrates that facilitate the administration of stem cells to target tissues in the context of stem cell therapy as well as to tools for manipulating and implanting such substrates into a target tissue.

2. Description of the Related Art

The scope of human disease that involves loss of or damage to cells is vast and includes, but is not limited to, ocular disease, neurodegenerative disease, endocrine diseases, cancers, and cardiovascular disease. Cellular therapy involves the use of cells, and in some cases stem cells to treat diseased or damaged tissues. It is rapidly coming to the forefront of technologies that are poised to treat many diseases, in particular those that affect individuals who are non-responsive to traditional pharmacologic therapies.

In fact, many diseases that are candidates for application of cellular therapy are not fatal, but involve loss of normal physiological function. For example, ocular diseases often involve functional degeneration of various ocular tissues which affects the vision, and thus the quality of life of numerous individuals.

The mammalian eye is a specialized sensory organ capable of converting incoming photons focused by anterior optics (cornea and lens) into a neurochemical signal. This process of phototransduction allows for sight by sending action potentials to higher cortical centers via the optic nerve. The retina of the eye comprises photoreceptors that are sensitive to various levels of light and interneurons that relay signals from the photoreceptors to the retinal ganglion cells. These photoreceptors are the most metabolically active cells in the eye (if not the body), and are supported metabolically and functionally by retinal pigmented epithelial cells (RPE). These RPE are positioned in a monolayer in the eye and are critical to vision.

Numerous pathologies can compromise or entirely eliminate an individual's ability to perceive visual images, including trauma to the eye, infection, degeneration, vascular irregularities, and inflammatory problems. The central portion of the retina is known as the macula, which is responsible for central vision, fine visualization and color differentiation. The function of the macula may be adversely affected by age related macular degeneration (wet or dry), diabetic macular edema, idiopathic choroidal neovascularization, high myopia macular degeneration, or advanced retinitis pigmentosa, among other pathologies.

Age related macular degeneration typically causes a loss of vision in the center of the visual field. Macular degeneration occurs in "wet" and "dry" forms. Taken together, these diseases affect approximately 1.75 million people in the U.S alone. The prevalence of those blinded by AMD is expected to increase to over 2.95 million by 2020. (See e.g., Friedman, D S et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol* 2004; 122:564-72.) In the dry form, cellular debris (drusen) accumulates between the retina and the choroid, the blood supply of the outer retina, due to the inability of diseased RPE cells of phagocytosing photoreceptor (PR) shed outer disc segments. Resulting hardened lipids (lipofuscin) impede the reciprocal exchange of nutrients and waste products between the retina and choroid, and lead to PR death. In the more severe wet form, newly formed blood vessels from the choroid infiltrate the space behind the macula. The walls of these newly formed vessels are mechanically weak, and extremely susceptible to rupture. Hemorrhage usually results in loss of vision extremely quickly compared with dry AMD. In conjunction with the loss of functional cells in the eye, the newly formed blood vessels are fragile and often leak blood and interstitial fluid, which can further damage the macula.

While diseases that cause damage to specific cells or tissues are clear candidates for cellular therapy, there remains a need in the art for methods, substrates, and tools to improve the efficacy of cellular therapy.



## SUMMARY

Many tissues are structurally or functionally dynamic in that the tissues flex during normal function, are subject to fluid flow or other shear stresses, or have numerous specialized cell types in close juxtaposition, thereby limiting the selection of target sites for cell delivery. As such, cellular therapy can require specific devices and methods to administer cells to a target tissue that enhance the activity and beneficial effects of the administered cells at the target tissue for an extended period of time. Depending on disease type, site of administration, advancement of pathology, and the type and time course of integration required between graft and host, devices and methods for cellular therapy require specifications which optimize safety and efficacy of the specific therapeutic.

In several embodiments, there is provided a substrate for cellular therapy to treat diseased or damaged ocular tissue, comprising a non-porous polymer having an apical and basal surface, wherein the substrate is configured to support a population of cells suitable for the treatment of diseased or damaged ocular tissue, and wherein, upon implantation into a subject, the substrate supports the population of cells for a period of time sufficient to treat the diseased or damaged ocular tissue.

In several embodiments, the substrate apical surface is substantially homogeneous and suitable for the growth of cells thereon. In some embodiments, the thickness of the substantially homogeneous apical surface ranges from about 0.1 to about 4 microns. In one embodiment, the thickness of the substantially homogeneous apical surface is between about 0.1 and about 0.5 microns. In some embodiments, the substantially homogeneous apical surface is roughened or otherwise treated to allow a population of cells to have a non-planar surface to grow on. The substantially homogeneous apical surface need not therefore be completely uniform in all embodiments. Rather, several embodiments comprise apical surfaces that are non-uniform, yet still substantially homogeneous. In several embodiments, the thickness of the substantially homogeneous apical surface for the growth of cells prohibits passage of proteins larger than about 60 kDa through the substrate. The thickness can be altered in different embodiments depending on the cell type and the size of proteins that are to be restricted (or allowed).

The tailored dimension allows for the passage of nutrients through the substrate (or the removal of metabolic byproducts through the substrate).

In several embodiments, the basal surface is inhomogeneous. In one embodiment, the basal surface comprises a plurality of supporting features juxtaposed with the apical surface. In one embodiment, a single continuous support feature is present. In other embodiments, multiple support features are provided. The support features provide sufficient structure to the substrate to allow for surgical implantation, but not so much rigidity that the substrate is kinked or otherwise creased during the implantation process. In several embodiments, the height of the supporting features ranges from about 3 μm to about 150 μm. In one embodiment, the supporting features ranges from about 3 μm to about 8 μm. In some embodiments, the substrate is oblong (e.g., longer than wide) and in some such embodiments, the supporting features run longitudinally along the long axis of the substrate. In this manner they provide structural rigidity along the long axis, but permit flexibility along the shorter axis. In other embodiments, the supporting features comprise columns of any appropriate geometric shape. In some embodiments, the substrate is round, in some embodiments, the substrate is square, and in some embodiments, the substrate is rectangular.

In one embodiment, the length and width of the substrate each range from about 0.3 mm to about 7 mm.

In several embodiments the outer edges and corners of the substrate are rounded, in order to minimize risk of damage to the substrate during implantation (e.g., by catching or snagging on tissue during implantation).

In some embodiments, the substantially homogeneous apical surface further comprises a raised lip surrounding the surface, wherein the raised lip has a height ranging from about 10 to about 1000 microns and a width ranging from about 10 to about 1000 microns. In several embodiments the lip serves not only to protect the cells growing on the substrate, but also to reduce trauma to the tissue during implantation.

In several embodiments, the non-porous polymer that comprises the substrate is non-biodegradable. In several embodiments, the non-porous polymer is selected from the group of consisting parylene A, parylene AM, parylene C, ammonia treated parylene, parylene X, parylene N, any of the foregoing polymers coated with polydopamine, any of the foregoing polymers coated with matrigel, any of the foregoing polymers coated with vitronectin, and any of the foregoing polymers coated with retronectin.

In one embodiment, the non-porous polymer is oxygen-treated. In one embodiment, the non-porous polymer is parylene C coated with one or more of matrigel, vitronectin, and retronectin. In one embodiment, oxygen-treated polymers are coated with one or more of matrigel, vitronectin, and retronectin (or combinations thereof). In other embodiments, alternatives to matrigel, vitronectin, and retronectin may also be used in place of or in addition to matrigel, vitronectin, and retronectin. Likewise, other modifying treatments (in addition to or in place of oxygen-treatment) may be employed.

In other embodiments, the non-porous polymer is a biodegradable polymer. In some such embodiments, the biodegradable polymer is selected from the group consisting of PLGA, polyethylene glycol modified and polycaprolactone.

In still additional embodiments, combinations of biodegradable and non-biodegradable polymers are used.

In several embodiments, the substrates disclosed herein are configured to support a population of retinal pigmented epithelial (RPE) cells. In one embodiment, retinal pigmented epithelial cells are human embryonic stem cell-derived RPE cells. In several embodiments, after seeding a substrate with RPE (or other stem cells), the cell-seeded substrate is suitable for implantation into the subretinal space of the eye of a subject, thereby treating an outer retinal degenerative disease. In several embodiments, the substrates are suitable for implantation in order to treat one or more outer retinal degenerative diseases (including, but not limited to) dry AMD, wet AMD, Stargardt's disease, and Leber's Congenital Ameurosis.

In several embodiments, there is also provided a substrate for cellular therapy to treat diseased or damaged ocular tissue, comprising a non-porous polymer, wherein the substrate comprises a substantially homogeneous apical surface for the growth of a population human embryonic stem cell-derived RPE cells, wherein the thickness of the substantially homogeneous apical surface ranges from about 0.1 to about 4 microns, wherein the substantially homogeneous apical surface is selected from the group consisting of parylene A, parylene AM, ammonia treated parylene, and parylene C, wherein the substrate comprises an inhomogeneous basal surface comprising supporting features juxtaposed with the substantially homogeneous apical surface, wherein the inhomogeneous basal surface comprises a polymer selected from the group consisting of parylene A, parylene AM, ammonia treated parylene, and parylene C, wherein one or more of the substantially homogeneous apical surface and the inhomogeneous basal surface is treated with one or more of matrigel, poly-L-dopamine, vitronectin, or retronectin, wherein the height of the supporting features ranges from about 3 μm to about 150 μm; and wherein, upon implantation into a subject, the substrate supports the population of cells for a period of time sufficient to treat the diseased or damaged ocular tissue. In some embodiments, one or more of the substantially homogeneous apical surface and the inhomogeneous basal surface are oxygen-treated.

In several embodiments, there is provided a substrate for cellular therapy to treat diseased or damaged ocular tissue, comprising a non-porous biodegradable polymer, wherein the substrate comprises a substantially homogeneous apical surface having a thickness from about 0.1 to about 4 microns for the growth of cells, wherein the substrate comprises an substantially homogeneous basal surface juxtaposed with the substantially homogeneous apical surface, wherein the substrate is configured to support a population of cells suitable for the treatment of diseased or damaged ocular tissue, and wherein, upon implantation into a subject, the substrate supports the population of cells for a period of time sufficient to treat the diseased or damaged ocular tissue.

In one embodiment, the substrate seeded with cells is suitable for implantation into the subretinal space of the eye of a subject. In one embodiment, the substrate is seeded with RPE cells, and wherein subsequent to implantation, the RPE cells on the substrate functionally interdigitate with the outer segments of the photoreceptors of the eye of the subject.

In several embodiments, there is provided a method of treating a subject having outer retinal degenerative disease, comprising: surgically positioning a substrate comprising a non-porous polymer having an apical and basal surface, wherein the apical surface is seeded with a population of cells into a position juxtaposed to the outer segments of the photoreceptors in the eye of the subject, thereby treating the degenerative disease. In several embodiments, the substrate is surgically positioned in the sub-retinal space or adjacent to the epiretinal side of the retina of an eye of the subject.

In several embodiments, the cells seeded on the substrate are RPE cells. In several embodiments, the RPE cells functionally and/or metabolically support damaged or diseased photoreceptors, thereby treating the degenerative disease. In some embodiments, the RPE cells functionally and metabolically support healthy photoreceptors as well. In several embodiments, the RPE support the photoreceptors through metabolically functional interdigitation with the outer segments of the photoreceptors.

In several embodiments, such methods are used to treat outer retinal degenerative diseases which include, but are not limited to dry AMD, wet AMD, Stargardt's disease, and Leber's Congenital Ameurosis, and retinitis pigmentosa.

In several embodiments, there is provided a substrate for cellular therapy to treat diseased or damaged ocular tissue comprising a non-porous polymer having a roughened apical surface topology for the growth of cells and an inhomogeneous basal surface comprising supporting features juxtaposed with the roughened apical surface topology, wherein the substrate is configured to support a population of cells suitable for the treatment of diseased or damaged ocular tissue, and wherein, upon implantation into a subject, the substrate supports the population of cells for a period of time sufficient to treat the diseased or damaged ocular tissue. In several embodiments, the substrate is fabricated in a substrate frame comprising a plurality of substrates.

In some embodiments, the thickness of the substantially homogeneous apical surface ranges from about 0.1 to about 4 microns. In several embodiments, the thickness of the substantially homogeneous apical surface for the growth of cells prohibits passage of proteins larger than about 75 kDa through the substrate. In some embodiments, the substantially homogeneous apical surface further comprises a raised lip surrounding the surface. In some such embodiments, the raised lip has a height ranging from about 10 to about 1000 microns and a width ranging from about 10 to about 1000 microns. Other embodiments do not have a lip. In some embodiments, the height of the supporting features ranges from about 3 μm to about 150 μm.

In several embodiments, the non-porous polymer is non-biodegradable while in other embodiments, the non-porous polymer is biodegradable. In some embodiments, the biodegradable polymer comprises polyethylene glycol modified polycaprolactone, PLGA, gelatin-modified silicone, or an anhydride polymer.

In several embodiments, the non-porous polymer is selected from the group consisting of parylene A, parylene AM, parylene C, ammonia and/or oxygen treated parylene C (for the purposes of adding functional groups and roughening the surface), and parylene C treated with either polydopamine, vitronectin, retronectin, or matrigel. In one embodiment, the non-porous polymer comprises parylene AM treated with polydopamine, and the inhomogeneous basal surface comprises parylene. In one embodiment, the substrate is configured to support a population of retinal pigmented epithelial (RPE) cells. In one embodiment, the retinal pigmented epithelial cells are human embryonic stem cell-derived RPE cells.

In several embodiments, the substrate is seeded on its substantially homogeneous apical surface with cells selected from the group consisting of: RPE cells, RPE and photoreceptors, Müller glial cells, ganglion cells, a mixture of Müller glial cells and ganglion cells, corneal endothelial cells, a mixture of corneal endothelial cells and collagen, corneal epithelial cells, a mixture of corneal epithelial cells and collagen, endothelial cells, pericytes, a mixture of endothelial cells and pericytes.

In several embodiments, the cell-seeded substrate is implanted in the subretinal space of the eye of a subject. In several such embodiments, the RPE cells interdigitate with the outer segments of the photoreceptors of the eye of the subject. In some embodiments, the RPE cells interdigitate with the outer nuclear layer of the photorecptors.

In some embodiments, the cell-seeded substrate is implanted adjacent to the epiretinal side of the retina of the eye of a subject.

In some embodiments, the cell-seeded substrate is implanted adjacent to corneal tissue of the eye of a subject.

In several embodiments, the cell-seeded substrate is suitable for cellular therapy for treatment of dry AMD, for treatment of corneal disease, for treatment of glaucoma, for treatment of diabetic retinopathy, for treatment of retinal vein occlusions, for treatment of wet AMD, and/or for treatment of retinitis pigmentosa. Other ocular diseases may also be treated with such substrates.

In several embodiments, there is provided a substrate for preparing cells for cellular therapy to treat diseased or damaged ocular tissue comprising a non-porous polymer comprising a substantially homogeneous apical surface for the growth of cells in an interconnected monolayer of cells, wherein prior to delivery to a subject, the interconnected monolayer of cells is detached from the substrate and the monolayer is implanted in the subject, thereby treating the diseased or damaged ocular tissue.

In several embodiments, there is provided a substrate for cellular therapy to treat diseased or damaged ocular tissue comprising a non-porous, permeable, non-biodegradable polymer selected from the group consisting of parylene A, parylene AM, ammonia etched parylene, and parylene coupled with polydopamine configured to form a roughened apical surface for the growth of cells, wherein the substantially homogeneous apical surface is coated with one or more of cyclic or linear arginine-glycine-aspartic acid or a synthetic growth matrix, and an inhomogeneous basal surface comprising supporting features juxtaposed with the substantially homogeneous apical surface. In several embodiments, the substrate is configured to support a population of cells suitable for the treatment of diseased or damaged ocular tissue and, upon implantation into a subject, the substrate supports the population of cells for a period of time sufficient to treat the diseased or damaged ocular tissue. In some embodiments, wherein the thickness of the substantially homogeneous apical surface ranges from about 0.1 to about 6 microns, and the height of the supporting features ranges from about 3 μm to about 150 μm.

In several embodiments there is provided a system for preparing a substrate for cellular therapy, comprising: a polymeric substrate frame comprising a plurality of individual substrates and a device for temporarily maintaining the substrate frame in a fixed position within a culture vessel.

In several embodiments the individual substrates are capable of being removed individually from the substrate frame. In some embodiments, the device is configured to prevent growth of cells on at least one portion of each of the plurality of individual substrates, and some embodiments the device is configured to allow selective removal of an individual substrate from the substrate frame.

In some embodiments of the system, the polymeric substrate frame and the individual substrates comprises a non-biodegradable polymer while in other embodiments, the polymeric substrate frame and the individual substrates comprises a biodegradable polymer.

In several embodiments the substrates are removed by cutting a portion of the substrate that connects the substrate to the substrate frame. Advantageously, several embodiments of the device configured to prevent growth of cells comprises an aperture through which the portion of the substrate can be cut.

In several embodiments, there is provided a method of treating a subject having outer retinal degenerative disease comprising surgically positioning an substrate as disclosed herein in a position juxtaposed to the outer nuclear layer of the photoreceptors in the eye of the subject. In several such embodiments, RPE cells seeded on the substrate support the photoreceptors, thereby treating the degenerative disease.

In several embodiments, there is provided a three-dimensional implantable substrate cage for cellular therapy to treat outer retinal degenerative disease. In some embodiments, the substrate cage comprises an outer shell having one or more pores therein and configured to form an inner lumen which is configured to receive stem cells. In some embodiments, the pores are configured to retain the one or more types of stem cells within the inner lumen while allowing an interaction between the one or more types of stem cells and cells of the target tissue.

In several embodiments, there is provided a method for treating retinal degeneration comprising culturing a plurality of stem cells, harvesting the stem cells, deploying the cultured stem cells into a three-dimensional polymeric substrate cage, implanting the cage into a target tissue.

In one embodiment, the outer shell of the substrate is polymeric. In one embodiment, the substrate further comprises a reporting feature. In some embodiments the reporting feature comprises microelectromechanical systems (MEMS) technology. In some embodiments, the MEMS reporting feature reports to a user regarding the viability of the cells housed within the substrate.

In one embodiment, the cells are RPE cells and the target tissue is the macula that has been damaged by age-related macular degeneration or other ocular disease. In one embodiment permeability of the substrate is defined solely by thickness of the biocompatible substrate. In one embodiment, the pores are between 0.5 and 1.5 μm. In one embodiment, the outer shell comprises polycaprolactone. In one embodiment, the polycaprolactone shell further comprises one or more of PEG and Arginine-Glycine-Asparagine. In one embodiment, the pores are generated by exposing the polymer substrate to an aqueous media.

In several embodiments, there is provided a method of fabricating a three-dimensional substrate cage for cellular therapy comprising preparing a substrate for the growth of cells, generating pores within the substrate, and sterilizing the substrate. In one embodiment, one or more types of stem cells are cultured on the substrate and a three-dimensional substrate cage is thereafter aligned and sealed, thereby containing the cells.

In several embodiments, there is provided a method of fabricating a three-dimensional substrate cage for cellular therapy comprising preparing two molds corresponding to top and bottom portions of the three dimensional substrate cage and configured to form an inner lumen upon assembly, polymerizing a polymeric solution in each portion of the mold, generating pores within the top and/or bottom portions of the substrate; and sealing the remaining portion or portions of the substrate cage with the substrate, thereby creating a three-dimensional substrate cage for cellular therapy. In one embodiment, the substrate cage is sterilized and sealed, and then cells are delivered into the inner lumen prior to implantation.

In one embodiment, the substrate cage is delivered to the subretinal space of an individual having retinal degenerative disease, and the substrate cage retains the stem cells the substrate cage after implantation but also allows processes from the retained cells to pass through the pores and interact with photoreceptors in the subretinal space. In one embodiment, the substrate cage is delivered to the subretinal space of an individual having retinal degenerative disease, and the substrate cage retains the stem cells after implantation but also allows chemical and cellular interaction between the encapsulated cells and the photoreceptors via the substrate cage pores. In one embodiment, the interaction supports the photoreceptors, thereby treating the retinal degeneration. In one embodiment, the interconnection further comprises one or more cells from the subretinal space of the individual passing through the pores and interacting with the substrate cage or the cells. In one embodiment, the substrate cage is implanted via an ab-interno surgical approach. In one embodiment, the substrate cage is implanted via an ab-externo surgical approach. In one embodiment, the individual afflicted with retinal degeneration is a mammal and in one embodiment, the mammal is a human. In one embodiment, the stem cells are deployed into the substrate cage immediately prior to implantation.



## BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A-**1**B depict general substrate structures in accordance with several embodiments disclosed herein.

FIGS. **2**A-**2**B depict internal views of several embodiments disclosed herein. FIG. **2**A depicts a side view of a substrate structure in accordance with several embodiments disclosed herein. FIG. **2**B depicts an internal view of a substrate cage structure in accordance with several embodiments disclosed herein.

FIG. **3** depicts a top cut-away view of a substrate in accordance with several embodiments disclosed herein.

FIG. **4** depicts a representation of an substrate cage with customizable chambers in accordance with several embodiments discloses herein

FIG. **5** depicts a representation of a patient's visual field used in fabricating a customized substrate cage according to several embodiments disclosed herein.

FIG. **6** depicts an example of a scotoma map used to construct a custom ocular substrate.

FIG. **7** depicts a mold shell used in several embodiments of fabrication of a custom substrate.

FIG. **8** depicts an example of a custom fabricated substrate constructed according to methods disclosed herein.

FIG. **9** depicts the loading of cells according to several embodiments disclosed herein.

FIG. **10** depicts a cross-section of a substrate in accordance with several embodiments disclosed herein.

FIGS. **11**A-**11**B depict the similarity between cultured hESC-derived RPE (FIG. **11**A) cells and adult RPE cells (FIG. **11**B).

FIGS. **12**A-**12**B depict characteristics of cultured hESC-RPE. FIG. **12**A shows scanning electron microscopy of polarized hESC-RPE showing apical specialization and microvilli. FIG. **12**B depicts phagocytic activity of hESC similar to that of human native fetal RPE.

FIGS. **13**A-**13**C depict visualization of post-implantation RPE cells. FIG. **13**A depicts rat fundus photograph showing PLGA-RPE one week after implantation in the subretinal space; FIG. **13**B depicts an OCT scan revealing the PLGA-RPE sheet (white arrow); FIG. **13**C depicts the OCT image through the non-transplant area.

FIGS. **14**A-B depict hESC-RPE transplanted into the subretinal space of RCS rats. FIG. **14**A depicts nuclear staining (DAPI) of preserved photoreceptors (ONL) in the transplanted area compared to the adjacent non-transplanted area (FIG. **14**B).

FIGS. **15**A-**15**B depict growth of cells on various substrates in accordance with several embodiments herein. FIG. **15**A depicts hESC-RPE on PLGA by scanning electron microscopy (cross section). FIG. **15**B shows growth of hESC-RPE surface modified parylene.

FIGS. **16**A-**16**B depict high resolution imaging SDOCT of the retina used in several embodiments disclosed herein.

FIGS. **17**A-**17**B depict top and side views, respectively, of a multi-chambered substrate cage comprising a substrate used to deliver cells in accordance with several embodiments herein. The substrate can be either non-degradable or degradable, facilitating interaction between cells in top and bottom chambers via defined material specifications. In some embodiments degradation rate and thickness of the material may be dependent on the time required for cells in both chambers to be implanted in the target location and make meaningful synaptic connections with, or allow for proper reciprocal nutrient exchange between, proximal cells.

FIGS. **18**A-**18**D depict various embodiments of substrate layouts in substrate frames.

FIGS. **19**A-**19**B depict top views of various substrate shapes in accordance with several embodiments disclosed herein.

FIG. **20**A-**20**F depicts various substrates disclosed herein and related permeability data. A side view of an asymmetric substrate having a homogeneous apical surface **100** and an inhomogeneous basal surface comprising supporting structures in accordance with several embodiments disclosed herein is shown in FIG. **20**A. Some embodiments further comprise a lip surrounding the apical surface **120** (see FIG. **20**B). Some embodiments further comprise a coating on the cell growth surface (FIGS. **20**C-**20**D). Manipulation of the thickness of the substrate allows tuning of molecular size exclusion and diffusion (FIGS. **20**E and **20**F, respectively).

FIGS. **21**A-**21**C depict various substrate embodiments disclosed herein. FIG. **21**A depicts a scanning electron microscopic image of the planar apical surface of one embodiment of a substrate described herein. FIG. **21**B depicts a scanning electron microscopic image of the bottom supporting surface of one embodiment of a substrate. FIG. **21**C depicts a growth of stem cells on the planar apical surface of a substrate.

FIG. **22**A depicts an additional embodiment of substrate layout in a substrate frame.

FIG. **22**B depicts one embodiment of a device used to hold a substrate frame and cut individual substrates from the frame.

FIGS. **23**A-**23**D depict histological results from implantation of a stem cell-seeded substrate implanted in a rat eye.

FIG. **24** depicts transmission electron microscopy of RPE cells implanted in the eye of a dystrophic rat at 9 days post-implantation.

FIG. **25** depicts transmission electron microscopy of RPE cells implanted in the eye of a dystrophic rat at 58 days post-implantation. Interdigitation is visible between the RPE microvilli and the photoreceptor outer segment disks.

FIG. **26** depicts transmission electron microscopy of RPE cells implanted in the eye of a dystrophic rat at 58 days post-implantation. Interdigitation is visible between the RPE microvilli and the photoreceptor outer segment disks.

FIG. **27** depicts scanning electron microscopy of RPE cells growing on the apical surface of a substrate in accordance with several embodiments disclosed herein.

FIGS. **28**A-**28**C depict fluorescent immunohistochemistry images of RPE cells seeded on a polymeric substrate in accordance with several embodiments disclosed herein.

FIGS. **29**A-**29**C depict additional fluorescent immunohistochemistry images of RPE cells seeded on a polymeric substrate in accordance with several embodiments disclosed herein.

FIG. **30** depicts optokinetic nystagmus (OKN) data collected from normal, untreated transgenic blind rats, and from treated transgenic blind rats after a bolus injection of RPE cells into the sub-retinal space of the eye of the rats.

FIG. **31** depicts OKN data collected from normal, untreated transgenic blind rats, and from treated transgenic blind rats after implantation of a substrate seeded with RPE cells into the sub-retinal space of the eye of the rats, as disclosed herein.

FIG. **32** depicts evidence of functional interdigitation (by detection of rhodopsin) between photoreceptors and RPE cells seeded on a substrate and implanted into the eye of a transgenic blind rat.

## DETAILED DESCRIPTION

Cellular therapy, the introduction of new cells into a tissue in order to treat a disease, represents a possible method for repairing or replacing diseased tissue with healthy tissue. Many approaches involve administration of cells (e.g., stem cells) to a target tissue, which often yields low retention rates and decreased incidence of long-term persistence of the transplanted (or implanted) cells. This may be due to a variety of factors, including cell washout and/or low cell survival rates in the delivery media. However, some diseases do not require the delivery or engraftment of the cells per se, but rather can require growth factors, chemical signals, or other interactions with the delivered cells. Several embodiments disclosed herein are directed to treating such diseases, and as such, comprise substrates configured to deliver the beneficial effects of cells (including physical, chemical, or other interaction) while retaining the cells within the substrate.

In particular, several embodiments relate to substrates into which stem cells are deposited in or on the substrate prior to administration of the substrate to a cell-therapy subject, and which function, post-administration, to retain the cells within or on the substrate while simultaneously facilitating a physical interaction between the stem cells within or on the substrate and portions of the target tissue. In several embodiments in particular, stem cells deposited within or on the substrate provide supportive effects for damaged or diseased cells of a target tissue, including, but not limited to, physical cell-cell interaction, release of nutrients or growth factors to the target tissue, attraction of other cell types, and the like. In several embodiments, the beneficial effects include one or more of secretion of growth factors which maintain the structural integrity of the choriocappilaris endothelium and photoreceptors (e.g. PEDF and VEGF), suppression of immunosuppressive factors which aids in the immune privileged status of the eye (which helps to suppress immune cell infiltration into the eye), secretion of neurotrophic factors, metabolic (e.g. exchange of glucose and fatty acids), functional benefits (e.g. delivery of retinol, phagocytosis of shed outer disc segments, and/or the reisomerization and restoration of visual pigments after photobleaching), or support of neural activity. In some embodiments, the support of neural activity occurs via interdigitation of the cells within or on the substrate with target tissue cells. For example, in one embodiment, apical microvilli of retinal pigmented epithelial cells within an implanted substrate interdigitate with host photoreceptors, thereby incurring a beneficial effect on the photoreceptors. In several embodiments, the benefit is via synapse formation (e.g. PR/bipolar cell), or other physical or chemical support of general cell viability). In several embodiments, one or more of these benefits occurs while partially or fully retaining cell somas within or on the substrate. In some embodiments, the cells from the host tissues project or infiltrate the substrate through the biological vias present in some embodiments of the substrate. For example, in some embodiments, new blood vessels or fibrous tissue protrude into the substrate. In some embodiments, these protrusions assist in anchoring the substrate, while in other embodiments, other beneficial effects (e.g., nutrient delivery, blood supply) are realized. Thus, as used herein, the term "interaction" shall be given its ordinary meaning and shall also refer to a one-way (implanted cells to target tissue or target-tissue to implanted cells) interconnect between cells or a two-way interconnect (both implanted cells to target tissue or target-tissue to implanted cells occur).

Further, in some embodiments, methods are provided for fabricating a custom substrate to treat a damaged or diseased tissue of an individual using tailored stem cell therapy.

In several embodiments, porous substrates for improved cellular therapy are provided. In several embodiments, the substrates provided are non-porous (e.g., do not have an orifice or via) but are permeable. Several such embodiments control the permeability of the substrate based on changes in thickness of the substrate. In some embodiments, substrates are both permeable and porous, with the permeability and porosity facilitating the interconnections between the implanted cells and the native cells. In several embodiments, the substrate is configured to receive cells and position the received cells in an optimal manner to facilitate regeneration of a damaged or diseased target tissue. In some embodiments, the substrates have specific characteristics (e.g., sizes, shapes, porosity) that retain the cells within the substrate, but simultaneously promote physical, chemical, or other interaction between the cells and target tissue. As used herein, the term "promote" shall be given its ordinary meaning and shall also be read to mean allow, enhance, permit, facilitate, foster, encourage, induce, and synonyms thereof. In some embodiments, the substrate is biodegradable, while in others, a non-biodegradable substrate is provided. In some embodiments the substrate is partly biodegradable and partly non-biodegradable. In several embodiments, fully non-biodegradable substrates are particularly advantageous, as their positioning relative to the implanted cells prevents access of immune cells into the transplant site and reduces risk of infection. Moreover, in several embodiments, a non-biodegradable substrate allows for identification and explant of transplanted cells should removal or replacement of the cells (e.g., additional "doses" of cells) be required. In several embodiments, the substrates herein are fabricated as a microelectromechanical system (MEMS). In several embodiments, the cells delivered to and retained within the substrate are stem cells. In several embodiments the substrate, and the cells retained therein are used to treat the damage associated with a disease, such as ocular degeneration, cardiac disease, vascular disease, and the like.

Substrates

As discussed above, the variety of diseases that lead to damage or loss of function of particular cell types is vast and represents an area of medicine in need of treatment approaches that go beyond typical surgical or pharmacological approaches. To address this need cellular therapy involves the use of cells, and in some cases fetal, umbilical cord, placenta-derived, adult, induced, or human embryonic stem cells and/or their partially or fully differentiated cellular derivatives to treat diseased or damaged tissues via replacement or regeneration. In several embodiments, substrates that improve the efficacy of cellular therapy are provided. As used herein, the terms "substrate" shall be given its ordinary meaning and shall also be used interchangeably with the term "implant" and/or "device", though it shall be appreciated that some embodiments described herein do not require implantation per se (e.g., those functioning as a "patch on a target tissue surface"). The contextual basis will make it clear to one of ordinary skill whether a particular embodiment is to be implanted within a target tissue. In addition, as used herein, the term "deliver" shall be given its ordinary meaning and shall also refer to the physical, chemical, or other type of interaction that the cells housed within the substrate provide to the target tissue without the release of cells from the substrate and/or engraftment of cells into the target tissue.

Types of Substrate

Based on the variety of diseases in which cellular therapy can be employed, a variety of different types of substrates may be advantageous, depending on the disease. In general, while many of the substrates disclosed herein inherently have three dimensions (e.g., a length, a width, and a height), some substrates disclosed herein are designed with particular attention being directed to one or more of these dimensions. For example, as discussed more fully below, several embodiments are referred to as 3-D substrate cages. In such embodiments, the substrate is sufficient to allow formation of at least one interior lumen. For example, in some embodiments, a cage structure with a purposefully designed 3-dimensional shape functions to provide one or more lumens or cavities within the substrate which functions to retain cells within the structure after delivery to a target site (while continuing to allow interactions between the cells and the target tissue). However, it shall be appreciated that the methods of fabrication, implantation, and uses disclosed herein shall be applicable to of any variety of substrates, devices, or implants described herein, unless otherwise expressly specified.

In several embodiments, the substrate is asymmetrical and inhomogeneous with distinct structural features on the apical and basal surfaces of the substrate. As used herein, the term "asymmetrical" and "substantially inhomogeneous" shall be given their ordinary meaning and shall also refer to substantially non-planar or variable surfaces. Likewise, as used herein, the term "substantially homogeneous" shall be given its ordinary meaning and shall also refer to surfaces which are largely or completely planar, or those which have minimally variable surfaces. The term substantially homogeneous shall not exclude certain accessory characteristics that cause a surface to not be completely planar. For example, the apical surface of the substrate is, in some embodiments, surrounded by a rim on the perimeter which is intended to protect cell monolayer integrity from shear force imparted during transplantation and to inhibit lateral cell proliferation outside of the boundaries defined by substrate (see, e.g., **120** in FIG. **20**B). While not completely planar, such embodiments are intended to be viewed as substantially homogeneous. In several embodiments, the basal surface has periodic polymeric supports or columns providing mechanical stability to the substrate thereby (1) facilitating handling during cell culturing, and loading onto custom tool and subsequent surgical implantation, and (2) shielding a thinner membrane and overlying cellular sheet from mechanical disruption from force imparted due to positive or negative air pressure.

As used herein, the terms "3-dimensional" and "3-D" shall be given their ordinary meanings and shall also refer to those devices resembling a cage (e.g., having one or more interior lumens or cavities). In light of such variability in the design of substrates disclosed herein, the disclosure below, unless otherwise specified shall be appreciated to be applicable to any such variety of substrate.

Dimensions

Based on the variety of diseases in which cellular therapy can be employed, and limitations of delivery of cells alone, several embodiments provide substrates for improved cellular therapy. In several embodiments, the disease to be treated or the cells to be targeted define, at least in part, the dimensions of the substrate. For example, in several embodiments substrates are dimensioned for implantation in particular target tissues, while in other embodiments, substrates are dimensioned for placement on or near a target tissue.

In several embodiments, substrates disclosed here are utilized in treatment of ocular disease. In such embodiments, certain substrate dimensions are utilized depending on the ocular tissue to be targeted. For example, a substrate to be implanted in the vitreal chamber would likely differ in dimension from a substrate to be implanted in the suprachoroidal space. In general, dimensions of certain ocular cavities, spaces, and tissue can be obtained from general knowledge of ocular anatomy. In certain embodiments, specific measurements are obtained from an individual to determine the specific dimensions required to fabricate a customized substrate.

In several embodiments, anchor features are fabricated into the substrate to allow secure and precise positioning of the substrate at a target site. In some instances, once the substrate is in place and the cells within or on the substrate have established an interconnect with the target cells, micro-movements of the substrate could damage and/or sever the interconnect, thereby reducing the therapeutic efficacy of the cells. Therefore, in several embodiments, anchor structures are attached and/or built in to the substrates disclosed herein. For example, in some embodiments, one or more holes are provided that allow the substrate to be sutured to target tissue. In some embodiments, a bioadhesive and/or an adhesive protein is used. In some embodiments, microhairs or a roughened surface of the substrate provide friction-based anchoring of the substrate. In some embodiments, MEMS features such as clamps or latches are used to grasp or otherwise connect the substrate to the target tissue. In some embodiments, the target tissue is dimensioned sufficiently to securely hold a substrate without the need for specialized anchors.

With reference to FIG. **1**, certain general dimensions are provided for 3-dimensional (e.g., cage) substrates according to several embodiments described herein. In several embodiments, the substrate **10** comprises a substrate body **20** and a substrate tail **30**. In some embodiments, the length of the substrate tail D**1** ranges from about 0.5 mm to about 5 mm. In some embodiments, the tail measures from about 0.5 to 1 mm, from about 1.0 to 1.5 mm, from about 1.5 to 2.0 mm from about 2.0 to 2.5 mm, from about 2.5 to 3.0 mm, from about 3.0 to 3.5 mm, from about 3.5 to 4.0 mm, from about 4.0 to 4.5 mm, from about 4.5 to 5.0 mm, and overlapping ranges thereof. In some embodiments, the substrate tail measures between about 0.7 to 1.3 mm, including 0.8, 0.9, 1.0, 1.1, and 1.2 mm. In several embodiments, the substrate tail, the substrate body (below) or the total dimensions of the substrate as a whole are such that forceps may be used to grasp and manipulate the substrate. At the same time, these dimensions are balanced with maintaining sufficient structure to the substrate that it is not easily bent, kinked, or otherwise damaged during handling or implantation.

In several embodiments, the substrate body is generally circular (see e.g., FIG. **1**A and FIG. **19**A). However, in some embodiments (see e.g., FIG. **1**B and FIG. **19**B), other shapes, including but not limited to rectangles, squares, ovals, and cylinders are used. With reference to FIG. **1**A, or other embodiments having a generally circular body, the radius of the substrate body measures from about 0.5 to about 5 mm. In some embodiments, the radius measures from about 0.5 to 1 mm, from about 1.0 to 1.5 mm, from about 1.5 to 2.0 mm from about 2.0 to 2.5 mm, from about 2.5 to 3.0 mm, from about 3.0 to 3.5 mm, from about 3.5 to 4.0 mm, from about 4.0 to 4.5 mm, from about 4.5 to 5.0 mm, and overlapping ranges thereof. In some embodiments, the substrate body radius measures between about 0.7 to 1.3 mm, including 0.8, 0.9, 1.0, 1.1, and 1.2 mm.

Similar dimensions are used in several embodiments not having a rounded or circular body. With reference to FIG. **1**B, D**1**A represents the length of the substrate, and in some embodiments, ranges from about 0.5 to about 5 mm. In some embodiments, the length measures from about 0.5 to 1 mm, from about 1.0 to 1.5 mm, from about 1.5 to 2.0 mm from about 2.0 to 2.5 mm, from about 2.5 to 3.0 mm, from about 3.0 to 3.5 mm, from about 3.5 to 4.0 mm, from about 4.0 to 4.5 mm, from about 4.5 to 5.0 mm, and overlapping ranges thereof. Dimension D**2**A represents the width of the substrate and may have similar measurements as D**1**A above.

With reference now to FIG. **2**A, which is a side view depicting the 3-dimensional structure of several embodiments disclosed herein, dimensions D**3** and D**4** represent the thickness of the substrate cage wall **30**. D**3** and D**4** have the same dimension in some embodiments, while in other embodiments, the dimensions vary between the two. In some embodiments, D**3** and/or D**4** measure between about 1.0 and 5.0 microns. In some embodiments, the thickness of the wall ranges from about 0.5 to 1 μm, from about 1.0 to 1.5 μm, from about 1.5 to 2.0 μm, from about 2.0 to 2.5 μm, from about 2.5 to 3.0 μm, from about 3.0 to 3.5 μm, from about 3.5 to 4.0 μm, from about 4.0 to 4.5 μm, from about 4.5 to 5.0 μm, and overlapping ranges thereof. In some embodiments, the thickness of D**3** and/or D**4** is ranges from about 1.5 to 2.5 μm, including 1.6, 1.7. 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, and 2.4 μm. In one embodiment, D**3** is maximally about 7 μm, which allows microvilli to penetrate the vias **60** fully. In one embodiment, D**4** is greater than D**3** in order to provide structural rigidity to the substrate cage. In some embodiments, one or more surfaces of the substrate cage is fabricated in a flexible "accordion" shape, to allow the substrate cage to expand upon delivery of cells to the lumen of the substrate cage. In some embodiments this expansion is made possible by using a highly elastomeric polymer. In some embodiments, the cell delivery solution comprises a larger overall volume than the volume of cells that are desired to be retained within the substrate cage. As such, the accordion shape of some embodiments, allows the substrate cage to expand to accommodate this excess fluid and then retract as the excess fluid passes through the pores of the substrate cage.

As shown in FIG. **2**A, the lumen **50** is an open space within the outer shell of the substrate cage that houses the cells that will provide a therapeutic effect to the target tissue. Depending on the cell type to be delivered, the quantity of cells to be housed within the substrate cage, and other limiting physical parameters of the target tissue, the height of the lumen D**6** can range from about 4 μm to about 75 μm. In some embodiments, D**6** measures from about 4 to 10 μm, from about 5 to 20 μm, from about 10 to 30 μm, from about 25 to 40 μm, from about 30 to 50 μm, from about 45 to 60 μm, from about 50 to 75 μm, from about 65 to 75 μm, or overlapping ranges thereof. In several embodiments, D**6** ranges from about 10 to about 50 μm. In some embodiments, the height D**5** of the lumen **50** is the same as the height of the membrane **70** that allows cell delivery to the lumen, but prevents cellular backflow. However, in some embodiments, depending on the overall shape of the substrate may vary, such that a first portion does not have the same dimension as a second portion.

The total height of the substrate cage D**5** is a function of D**3**, D**4** and D**5**, as well as any characteristics of the target tissue that need to be accounted for in the fabrication of the substrate cage. In some embodiments, D**5** ranges from about 6 to about 85 μm, including 5 to 20 μm, 20 to 30 μm, 30 to 40 μm, 40 to 50 μm, 50 to 60 μm, 60 to 70 μm, 70 to 85 μm and overlapping ranges thereof. In some embodiments, the dimensions are adjusted to account for the location of the target tissue. For example, if the target tissue is frequently under load (e.g., pressure, contractile, etc.) but presents a small area suitable for implantation, the substrate cage can be fabricated with thicker walls and a suitable overall height (larger D**3** and/or D**4** relative to D**5**). As a result, D**6** would be smaller. In other embodiments, walls of the substrate cage can be fabricated to maximize D**6** relative to D**5**. Thus, in several embodiments, the dimensions of the substrate cage are tailored to the target tissue generally, or in some embodiments, to the target tissue dimensions of a particular individual.

With reference to FIGS. **18**A and **22**A, in several embodiments, a plurality of substrates are positioned within a culture vessel in order to allow for the concurrent seeding of cells across the plurality of substrates. In several embodiments, the diameter D**8** is designed to be slightly smaller than commercially available cell culture dishes adequate for cGMP scale up such that the substrate frame fits snuggly in the dish (e.g., fit to reduce/minimize movement of the substrate). Based on the diameter D**8** (or width, if not circular) of the substrate frame culture vessel, the number of substrates that are concurrently seeded with cells will vary. In some embodiments, and depending on culture dish diameter, D**8** is about 14-15 cm, about 9-10 cm, or about 4-5 cm. In some embodiments, D**8** is less than about 4 cm, including 3, 2, 1, and 0.5 cm. In several embodiments, multiwell culture vessels are used. For example, in several embodiments 6-well, 12-well, 24-well, or 48-well plates are used. In some embodiments, 96-well plates are used. In several embodiments, the depth of the wells ranges from about 1 to about 1.7 mm, including about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6 mm. In several embodiments, the diameter of the wells (which can be optimized based on the number of substrates to be cultured in the well) ranges form about 3 to about 20 mm, including about 3 to about 5 mm, about 5 to about 7 mm, about 7 to about 9 mm, about 9 to about 11 mm, about 11 to about 13 mm, about 13 to about 15 mm, about 15 to about 17 mm, about 17 to about 19 mm, about 19 to about 20 mm, and overlapping ranges thereof. In several embodiments, the height of the sidewalls ranges from about 10-20 mm, including about 10 to about 12 mm, about 12 to about 14 mm, about 14 to about 16 mm, about 16 to about 18 mm, about 18 to about 20 mm, and overlapping ranges thereof. Certain embodiments, of such culture vessels are commercially available with established well dimensions.

In some embodiments, a culture vessel that is rectangular or square is used in order to maximize the culture area of the vessel. In some embodiments, and especially with regard to rectangular or square dishes, a custom sized (e.g., not commercially available) culture vessel is designed and fabricated. Specifications of these custom dishes such as width and length are defined by multiple variables, including, but not limited to, (1) the minimum number of substrates or custom tool heads required to provide adequate sampling at final release testing to show lot-to-lot consistency, (2) finalized substrate shape (either circular (FIG. **19**A) or rectangular (FIG. **19**B)) and associated lateral dimensions (D**9**-D**11** with regard to circular embodiment; D**10**, D**12**, D**13** with regard to oblong or rectangular embodiment with rounded edges, or a oval shaped hybrid of the two), etc.

Based on the dimensions of the culture vessel, and the target tissue for the substrate, the dimensions of the substrates may vary. As discussed in more detail below and shown generally in FIG. **19**A, in several embodiments, there is provided a substantially inhomogeneous substrate having a circular body and a tail. The dimensions of this type of substrate, while overlapping with the 3-dimensional substrate cages described above, are specifically designed with the strength, durability, and manipulability of substrates that do not have a naturally increased stability by being formed in a cage-like structure or do not require a lumen. In some embodiments, a fully planar substrate is used. In some embodiments, both top and bottom surfaces are non-planar.

As such, the diameter D**9** of such inhomogeneous substrates ranges from about 1 mm to about 8 mm, including about 1 to about 2 mm, about 2 to about 3 mm, about 3 to about 4 mm, about 4 to about 5 mm, about 5 to about 6 mm, about 6 to about 7 mm, about 7 to about 8 mm, and overlapping ranges thereof. In certain embodiments, diameters of about 3 to about 5 mm are used, including 3.2, 3.4, 3.6, 3.8, 4.0, 4.2, 4.4, 4.6, 4.8, and 5.0 mm.

Tail (or handle) width D**10** ranges, in several embodiments, from between about 0.1 mm to about 6 mm, including about 0.2 to about 1.0 mm, about 1.0 to about 2.0 mm, about 2.0 to about 3.0 mm, about 3.0 to about 4.0 mm, about 4.0 to about 5.0 mm, about 5.0 to about 6.0 mm, and overlapping ranges thereof.

Tail (or handle) length D**11** ranges, in several embodiments, from between about 1 mm to about 20 mm, including about 1 to about 2 mm, about 2 to about 3 mm, about 3 to about 4 mm, about 4 to about 5 mm, about 5 to about 6 mm, about 6 to about 7 mm, about 7 to about 8 mm, about 8 to about 9 mm, about 9 to about 10 mm, about 10 to about 11 mm, about 11 to about 12 mm, about 12 to about 13 mm, about 13 to about 14 mm, about 14 to about 15 mm, about 15 to about 16 mm, about 16 to about 17 mm, about 17 to about 18 mm, about 18 to about 19 mm, about 19 to about 20 mm, and overlapping ranges thereof.

Similar tail width and length are used in oblong (e.g., oval or largely rectangular) substrates, as depicted generally in FIG. **19**B. Width D**12** of such substrates ranges, in several embodiments between about 0.2 and about 7 mm, including from about 0.2 to about 0.4 mm, about 0.4 to about 0.6 mm, about 0.6 mm to about 0.8 mm, about 0.8 mm to about 1.0 mm, about 1.0 to about 2.0 mm, about 2.0 to about 3.0 mm, about 3.0 to about 4.0 mm, about 4.0 to about 5.0 mm, about 5.0 to about 6.0 mm, and overlapping ranges thereof.

The length D**13** of such oblong substrates ranges, in several embodiments, from between about 0.5 mm to about 9 mm, including about 0.5 to about 1.0 mm, about 1.0 to about 2.0 mm, about 2.0 to about 3.0 mm, about 3.0 to about 4.0 mm, about 4.0 to about 5.0 mm, about 5.0 to about 6.0 mm, about 6.0 to about 7.0 mm, about 7.0 to about 8.0 mm, about 8.0 to about 9.0 mm and overlapping ranges thereof. In some embodiments, length D**13** ranges from about 4 to about 7 mm, including 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 6.8, and 7.0 mm.

With reference to FIG. **20**, certain general cross-sectional dimensions are provided for inhomogeneous substrates with and without a perimeter lip (FIG. **20**B). Embodiments employing either of these cross-sectional structures can be used with commercially available cell culture dishes, or other types of dishes such as those described above. In several embodiments, the substrate has a homogeneous apical layer **100** which provides a surface for cellular growth. In several embodiments, the thickness D**14** of the apical cell growth surface **100** is less than about 4 μm thick. In some embodiments, the thickness ranges from about 0.05 to about 0.1 μm, from about 0.1 to about 0.2 μm, from about 0.2 to about 0.5 μm, from about 0.5 to about 3.5 μm, from about 0.5 to about 3.0 μm, from about 0.7 to about 3.0 μm, from about 1.0 to about 3.0 μm, from about 1.5 to about 2.5 μm, from about 1.8 to about 2.2 μm, and overlapping ranges thereof. In some embodiments, the thickness ranges from between about 0.5 and 1.0 μm, including 0.6, 0.7, 0.8, and 0.9 μm. In certain embodiments, a thicker apical cell growth surface is used. For example, in some embodiments, the apical cell growth surface ranges from about 2 to about 3 μm, about 3 to about 4 μm, about 4 μm to about 5 μm, about 5 μm to about 6 μm, and overlapping ranges thereof. Depending on the target tissue, and on the specific region of the target tissue, and the cell type, thicker or thinner apical surfaces may be also used. As shown, the apical cell growth surface **100** is paired with thicker supports **110** on the basal surface. The supports **110** provide in some embodiments, mechanical rigidity, which aids in supporting the full apical cell growth surface during handling and surgical insertion. In those embodiments wherein the cells growing on the surface are RPE cells, after surgical insertion, the apical cell growth surface (and the RPE cells) will be juxtaposed with the photoreceptors in the eye, thereby serving to support the health of the photoreceptors. In several embodiments, the supports also inhibit lateral growth of the cells and possible extension of growth onto the basal side of the substrate. The supports, in several embodiments also inhibit cell growth into the vitreous via mechanical disruption of retinal integrity. (The latter results in a complication known as proliferative vitreo-retinopaty (PVR)). Therefore, such embodiments have a homogeneous apical surface conducive to cell growth paired with a basal surface having an inhomogeneous, but periodic, topology defined by the size and pitch of the supports. In some embodiments, the supports are columnar in shape, while in other embodiments, other shapes are used (e.g., cuboidal). In some embodiments a rim is employed on the apical surface to inhibit lateral growth and protect from shear force during surgical implantation.

As shown in FIGS. **20**A and **20**B, the basal surface supports **110** range in total height D**15** (as measured from the apical cell growth surface layer to the termination of the support) between about 3 μm and about 15 μm, including about 3 to about 5 μm, about 5 to about 7 μm, about 7 to about 9 μm, about 9 to about 11 μm, about 11 to about 13 μm, about 13 to about 15 μm, and overlapping ranges thereof. In some embodiments, the supports range in height from between about 5 μm to about 8 μm, including 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 6.4, 6.6, 6.8, 7.0, 7.2, 7.4, 7.6, 7.8, and 8.0 μm. Depending on the embodiment, larger supports (e.g., greater height) or smaller (e.g., lesser height) may also be used. For example, in several embodiments, the height of the supporting features ranges from about 5 μm to about 500 μm, including about 5 μm to about 50 μm, about 50 μm to about 100 μm, about 100 μm to about 150 μm, about 150 μm to about 200 μm, about 200 μm to about 300 μm, about 300 μm to about 400 μm, about 400 μm to about 500 μm, and overlapping ranges thereof. Likewise, in several embodiments the height of the supporting features ranges from about 1 μm to about 5 μm, including about 1 μm to about 1.5 μm, about 1.5 μm to about 2.0 μm, about 2.0 μm to about 2.5 μm, about 2.5 μm to about 3.0 μm, about 3.0 μm to about 3.5 μm, about 3.5 μm to about 4.0 μm, about 4.0 μm to about 5.0 μm, and overlapping ranges thereof.

In several embodiments, the apical and basal surfaces comprise different materials. For example, in one embodiment, the apical cell growth surface may be made of first material that is more conducive to cell growth, while the basal surface is made of a second (or modified first material) that imparts strength and/or durability to the substrate as a whole. In some embodiments, the apical surface comprises a coating or thin layer of deposited material **100'** that assists in cell growth and/or adherence. (see e.g., FIGS. **20**C and **20**D). In several embodiments, this layer is between about 10 and 100 nm in thickness. In some embodiments, the layer is between about 20 to 30 nm, between about 30 to 40 nm, between about 40 to 50 nm, between about 50 to 60 nm, between about 60 to 70 nm, between about 70 to 80 nm, between about 80 to 90 nm, between about 90 to 100 nm, and overlapping ranges thereof.

In several embodiments, the layer **100'** comprises parylene AM. In several embodiments the layer comprises ammonia treated parylene C. In several embodiments, the layer comprises parylene C and polydopamine. In several embodiments, parylene AM, parylene C, ammonia and/or oxygen treated parylene C (for the purposes of adding functional groups and roughening the surface), and parylene C treated with either polydopamine, vitronectin, retronectin, or matrigel are used. In several such embodiments, the second layer (**100** in FIGS. **20**A-**20**D) comprises a blend of cyclic and linear arginine-glycine-aspartic acid residues. In several embodiments, the second layer comprises a synthetic cell growth matrix (e.g., SYNTHEMAX™ by Corning). In one embodiment, the inhomogeneous basal surface comprises parylene C, with a parylene AM layer as the substantially homogeneous apical surface. In one embodiment, the parylene AM is coated with one or more of matrigel, vitronectin, retronectin, poly-L-dopamine. In one embodiment the substrate comprises parylene C, which is coated and/or treated directly. In several embodiments the poly-L-dopamine coating is generated by reacting PEG-(N-Boc-L-$DOPA)_2$) with cyclic Arginine-Glycine-aspartic in an oxidative aqueous media (such as sodium periodate ($NaIO_4$) to generate a poly-L-dopa coating. Alternatively, PEG-(N-Boc-L-$DOPA)_2$) is reacted with RGD-L-DOPA in an oxidative aqueous media (such as sodium periodate ($NaIO_4$) to generate a poly-L-dopa coating. In several embodiments the substrate comprises parylene C, which is coated and/or treated directly. In one embodiment, the substrate is coated with Matrigel, retronectin, vitronectin, equivalents thereof, and/or combinations thereof. Moreover, in several embodiments, a coating on the surface of the substrate is used in conjunction with another surface treatment method, such as, for example oxygen treating the substrate surface (disclosed in more detail below).

Alternative embodiments, a non-limiting example of which is shown in FIG. **20**B, further comprise a substrate lip **120** lining the perimeter of the apical cell growth surface **100** that functions to shield cells growing on the apical surface from fluid shear stress during culturing of the cells, manipulation of the substrate, and/or during or after surgical implantation into a target tissue (e.g., the eye). The lip further functions to define a boundary to control growth of the cells on the surface; thereby preventing the growth of the cells from the apical surface (cell growth surface **100**) to the basal side of the substrate. Moreover, the substrate lip **120** limits disruption of monolayer integrity during the separation of an individual substrate from an array of substrates (e.g., those shown in FIG. **18**A or **22**A). In several embodiments the lip has a width D**18** ranging from between about 10 μm to about 1500 μm (1.5 mm). In some embodiments, the width ranges from about 10 to about 100 μm, from about 100 to about 200 μm, from about 200 to about 300 μm, from about 300 to about 400 μm, from about 400 to about 500 μm, from about 500 to about 600 μm, from about 600 to about 700 μm, from about 700 to about 800 μm, from about 800 to about 1000 μm, from about 1000 to about 1100 μm, from about 1100 to about 1200 μm, from about 1200 to about 1300 μm, from about 1300 to about 1400 μm, from about 1400 to about 1500 μm, and overlapping ranges thereof.

In several embodiments the lip has a height D**19** ranging from between about from between about 10 μm to about 1500 μm (1.5 mm). In some embodiments, the height ranges from about 10 to about 100 μm, from about 100 to about 200 μm, from about 200 to about 300 μm, from about 300 to about 400 μm, from about 400 to about 500 μm, from about 500 to about 600 μm, from about 600 to about 700 μm, from about 700 to about 800 μm, from about 800 to about 1000 μm, from about 1000 to about 1100 μm, from about 1100 to about 1200 μm, from about 1200 to about 1300 μm, from about 1300 to about 1400 μm, from about 1400 to about 1500 μm, and overlapping ranges thereof. In some embodiments, the height ranges from between about 20 to about 300 μm, including about 20 to about 50 μm, about 50 to about 100 μm, about 100 to about 150 μm, about 150 to about 200 μm, about 200 to about 250 μm, about 250 to about 300 μm, and overlapping ranges thereof.

As shown in FIGS. **21**A-**21**C, such substrates having an apical cell growth surface and basal structural support surface can be fabricated by the methods disclosed herein and support cellular growth. FIG. **21**A depicts a scanning electron microscopy image of the apical cell growth surface of an substrate in accordance with the above description. As shown, the apical cell growth surface is homogeneous, while, as shown in FIG. **21**B, the basal surface is inhomogeneous, comprising multiple support structures. Further, as shown in FIG. **21**C, the apical cell growth surface is suitable for the growth of cells, e.g., stem cells (shown are H9 hESC-RPE cells proliferating on a parylene growth surface).

As discussed above, the particular tissue that is damaged or diseased and is to be targeted with a cell-loaded substrate may define the dimensions or structural features of the substrate. For example, a substrate used to target cells to the surface of an individual's liver could be fabricated with much larger dimensions than those described above. Similarly, a substrate fabricated to target the cardiac tissue of a patient could also be designed with larger overall dimensions. However, some substrates, such as those to target neural tissues, may benefit from dimensions more similar to those described above. Given the anatomical knowledge of those skilled in the art, dimensions for various target tissues can be readily obtained, or as discussed herein, specifically measured for a certain individual.

Porosity and Permeability

With reference to FIGS. **1** and **2**, in several embodiments, the substrate cages comprise porous materials. In some embodiments, the material is a permeable material. As shown, pores **60** are present in the outer shell of the substrate cage. In some embodiments, pores are present on the top **30***a* and bottom **30***b* surfaces of the substrate cage. As used herein, the term pore shall be given its ordinary meaning and also refer to "biological vias", in the sense that they function as passageways to allow the physical, chemical, or other types of interactions and/or interconnections described herein. The terms "pore" and "via" shall be read as interchangeable unless contextually indicated otherwise. In some embodiments, the vias on the top side of the substrate cage allow the passage of apical microvilli from cells contained within the substrate cage, while vias on the bottom side provide support for the cell bodies. In some embodiments, the density of pores on the top and bottom sides is similar, while in other embodiments, the pore density is different.

In several embodiments, pore density ranges between about $1\times10^6$ and $2.5\times10^9$ pores per $cm^2$. In some embodiments, pores have a distance between them of approximately 0.2 microns and about 2 micron center-to-center pitch. Greater or lesser spacing and pitch is used in other embodiments. In several embodiments, the parameters described above (pore diameters, density, and substrate thickness) affect the hydraulic conductivity and diffusion rate of nutrients and macromolecules across the substrate. In one embodiment, net flux across both substrates is zero. As such, in several embodiments, the bottom surface has a higher pore density compared with the top. To this end a dual layer photolithographic process flow allowing for decreased allowable effective pore diameter defined by sacrificial layer thickness can be employed for fabrication of the basal substrate. In some such embodiments, a first layer of material is fabricated with passages that communicate with either surface of the layer. This results, in cross-section, in a "block-like" pattern. See e.g., FIG. **10**. There after a second layer is fabricated, again with similar passages. In order to maintain the desired pore diameter, the second layer is photolithographically laid onto the first layer with a known gap size **60** that corresponds to the desired size of the biological via.

In still other embodiments, vias are present only on one of the sides, e.g., top or bottom. In some embodiments, although not expressly shown in the Figures, the apical and/or basal surface further comprises a separate substrate material that is annealed to the apical or basal surface during the fabrication process. In several embodiments, the vias range in diameter from about 0.5 to about 10 μm. Pore diameter may vary depending on the cell type to be housed within the substrate cage, the site of implantation, or the target tissue. In several embodiments, the pore diameter ranges from about 1 to 3 μm, 3 to 5 μm, 5-7 μm, 7 to 10 μm, or overlapping ranges thereof. In several embodiments, the pore diameter ranges from about 0.5 to about 1.5 μm, including 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, and 1.4 μm. While in several embodiments, the pore diameter is sufficiently large to allow cellular process to reach out of the substrate cage to the target tissue, the pore diameter is not large enough to allow the cell itself to escape the substrate cage. Thus, via diameter serves to retain the cells within the substrate cage, but allows the therapeutic effect of the cells to reach the target tissue, whether this be a physical (e.g. between a cellular process and the target tissue), chemical, or other type of interaction. However, in some embodiments, substrate cages are designed to let at least a portion of the cells within the substrate cage escape.

In several embodiments, the pore diameter is varied across the surface of the substrate cage during the fabrication process. In several embodiments, pore size may be varied to allow cells at a certain position in the lumen of the substrate cage to escape while other cells are retained within the substrate cage. In some embodiments, the substrate cage is fabricated with multiple chambers, and pore size may vary depending on the chamber the pore is in communication with. For example, a first chamber may house a first cell type to be retained within the substrate cage and provide a therapeutic effect to the target tissue. In such embodiments, the first chamber would be fabricated with a pore size that retained the cells within the substrate cage. See, for example FIG. **2**B chamber **50***a* and pore **60***a*. However, a second cell type may be housed within a second chamber, the second cell type providing an ancillary effect to the first cell type and/or the target tissue upon escape from the substrate. Thus, in such embodiments, the second chamber would be fabricated with pores of a diameter sufficient to allow the second cell type to escape the substrate cage. See, for example, FIG. **2**B, chamber **50***b* and pore **60***b*. In other embodiments, different chambers may house drugs or other ancillary agents, and are therefore fabricated with a porosity defined by the required or desired release rate of the drug or agent. In some embodiments, additional chambers are not required for a drug or ancillary agent. The drug or ancillary agents are used to support the viability of the cells, promote the interaction of the cells with the target tissue, inhibit or promote vascularization of the substrate or tissue near the substrate (depending on the target tissue) or other additional effects that potentiate or otherwise enhance the therapeutic effects of the cells on the target tissue.

With reference to FIG. **4**, different chambers **50**, **50***a*, **50***b*, and **50***c* may also be fabricated in a concentric or semi-concentric manner. The individual chambers are created by dividers **90**, **90***a*, and **90***b*. In several embodiments, the dividers comprise the same material as the body of the substrate cage. In several embodiments, dividers are constructed of a different material from the body of the substrate cage. In some embodiments, the dividers comprise wires through which an electrical current may be passed to create and seal off a chamber from the remainder of the lumen of the substrate cage. In other embodiments, the materials that generate the chambers may be altered to allow for visualization of the substrate cage in situ. For example, **90**, **90***a*, and **90***b*, may comprise a ring of chromium or other compound that allows visualization. These structures may also function as connectors for chemical sensors that allow reporting of information regarding the environment around the substrate (below). Further, they could function as a mechanism to secure an apical and basal portion of an substrate to one another, either before or after the seeding of the cells.

In several embodiments, the substrates are non-porous, e.g., no orifice or via exists that passes through the thickness of the substrate material. While lacking a specific passageway for passage of nutrients or cell processes, non-porous substrates have a permeability that can be manipulated based on the thickness of the material used. For example, the thickness of the substrate material allows the substrate to act as a molecular sieve, keeping certain proteins of a certain size from passing through the substrate, while allowing passage of proteins of other sizes through the substrate. See FIGS. **20**E and **20**F that depict data related to the diffusion of molecules (dextran) through substrates ranging from 0.15 to 0.80 μm in thickness. Diffusion coefficients (related to thickness) range from between about $10^{-10}$ and $10^{-13}$ $cm^2$/second. In some embodiments, greater or lesser rates of diffusion can be achieved by manipulating the thickness of the substrate.

As discussed above, several asymmetrical, inhomogeneous substrate embodiments (those with an apical cell growth surface and an inhomogeneous basal surface) are non-porous, but are permeable. A certain degree of permeability of the substrate material is necessary to support the cells grown on the substrate such that they are both metabolically and functionally viable over time (e.g., both in culture and post-implantation). For example, in several embodiments, parylene substrates having a thickness (e.g., D**14** in FIGS. **20**A-**20**) less than about 0.80 μm have a molecular weight exclusion limit of about 70-75 kDa. Such a substrate would exclude proteins or molecules larger than about 70-75 kDa, which would allow for the passage of most proteins present in the bloodstream that would be necessary to support cells on the substrate after implantation. In some embodiments, greater or lesser molecular exclusion can be achieved by manipulating the thickness of the substrate (e.g., exclusion limits ranging from about 25 kDa to about 150 kDa, including about 25 to about 30 kDa, about 30 to about 35 kDa, about 35 to about 40 kDa, about 40 to about 45 kDa, about 45 to about 50 kDa, about 50 to about 55 kDa, about 55 to about 60 kDa, about 60 to about 65 kDa, about 65 to about 70 kDa, about 70 to about 75 kDa, about 75 to about 100 kDa, about 100 to about 125 kDa, about 125 to about 150 kDa, and overlapping ranges thereof.

While it is appreciated in the art that delivery of nutrients to implanted (and existing cells) is important for the viability of the cells, it is particularly advantageous that certain embodiments of the substrates disclosed herein do not require a pore or an orifice to provide such nutrients. For example, wet AMD involves anomalous neovascularization by vessels that have mechanically weaker walls. This fragile vasculature risks rupture, subsequent hemorrhage, and the rapid loss of vision due to cell death. Such rupture could be compounded by the implantation of substrates having pores, through which such fragile vessels could grow. While dry AMD is non-neovascularizing disease, in some instances, the growth of vessels through the pores of a substrate cage could result in damage to the vessels, or disruption of the cells within a substrate cage. As discussed above, certain embodiments comprise non-porous substrate cages that are permeable to nutrients from the bloodstream. In such embodiments, pores in the substrate cage as well as the growth of vessels into such pores, is not possible, thereby limiting the possibility of blood vessel rupture and/or disruption of the cells growing on the substrate cage.

In some embodiments, certain materials may be used that are both permeable and porous. Selection and/or adjustment of the formulation of selected materials (e.g., co-polymers) are used, in some embodiments, to tailor the permeability and/or the porosity of the materials (and the resulting substrate).

Materials

A variety of materials may be used to fabricate the substrates disclosed herein. In some embodiments, the substrates are biodegradable while in other embodiments, the substrates are non-biodegradable. In still other embodiments, a portion of the substrate is biodegradable while another portion is not. In several embodiments, the biodegradable portions of the substrates can be fabricated to degrade at a known rate. Such biodegradable materials include any suitable material that degrades or erodes over time when placed in the human or animal body. Accordingly, as the term is used herein, biodegradable material includes bioerodible materials.

In several biodegradable embodiments, the materials selected are optimized to accomplish a particular rate of biodegradation. For example, in several embodiments employing polymers, the composition of the polymers is controlled to achieve a certain rate of biodegradation, and hence residency time of the substrate in vivo. By way of example, in one embodiment in which the substrate comprises a PLGA copolymer, the rate of biodegradation of the PLGA copolymer is controlled by varying the ratio of lactic acid to glycolic acid units in the copolymer. In some embodiments, the rate of biodegradation is controlled to achieve a residency to of approximately 4 weeks post-implantation. In some embodiments, the substrate degrades in about 1-3 weeks, 2-4 weeks, or longer, including from about 4-6 weeks or several months.

In addition to the materials used to fabricate the substrate itself, several embodiments comprise an additional biodegradable layer or coating that functions to delay the interaction between the target tissue and the cells housed within or on the substrate for a known period of time. For example, a coating with a rapid rate of degradation could be used to encapsulate the substrate and thereby protect the cells within or on the substrate during the implantation process and/or prevent the pores of the substrate from becoming obscured or blocked with tissue during the implantation process. Upon completion of implantation, the layer would rapidly degrade and the interaction between the cells within or on the substrate and the target tissue would commence. In contrast, in some embodiments, a more slowly degrading coating may be used. For example, if the implantation procedure was performed as a surgical procedure (or in conjunction with a surgical procedure), post-surgery medications (e.g., anti-inflammatories and/or antibiotics) which may adversely affect the cells within or on the substrate may be present for an extended period of time at or near the target tissue. In such cases, the degradation rate of the coating could be tailored to prevent the exposure of the cells to the target tissue until a time when the harmful agent was no longer present.

Some embodiments comprise a non-biodegradable material combined with a biodegradable material, the latter which provides additional structural and mechanical support aiding in substrate handling during cell seeding and culturing and/or during surgical insertion into a tissue (e.g., the subretinal space). The material may also be used to add mass to the substrate to assist in the same and/or to assist in orientation of the substrate. In several embodiments, the support is in the form of columns connecting the top and bottom portions of a substrate cage or on the basal portion of an inhomogeneous substrate. In other embodiments the support comprises an additional layer on the top or bottom of the cage or the basal surface of an inhomogeneous substrate.

The substrates may be formed of metals, polymers, plastics, or combinations thereof. In some embodiments, the material allows the substrate to have sufficient elasticity, flexibility and potential elongation to not only conform to the target anatomy during and after implantation, but also remain unkinked, untorn, unpunctured, and with a patent lumen during and after implantation. In several embodiments, substrate material would advantageously be processable in a practical manner, such as, for example, by molding, extrusion, thermoforming, and the like, as well as by the MEMS manufacturing methods discussed below.

The purpose of surface modification, in some embodiments, is to promote cell viability and attachment. This is done by functionalizing the surface. Towards this end, illustrative examples of suitable materials for the substrate include parylene polypropylene, polyimide, glass, nitinol, polyvinyl alcohol, polyvinyl pyrolidone, collagen, chemically-treated collagen, polyethersulfone (PES), poly(glycerol-sebacate) PGS, poly(styrene-isobutyl-styrene), polyurethane, ethyl vinyl acetate (EVA), polyetherether ketone (PEEK), Kynar (Polyvinylidene Fluoride; PVDF), Polytetrafluoroethylene (PTFE), Polymethylmethacrylate (PMMA), Pebax, acrylic, polyolefin, polydimethylsiloxane (PDMS) and other silicone elastomers, polypropylene, hydroxyapetite, titanium, gold, silver, platinum, other metals and alloys, ceramics, plastics and mixtures or combinations thereof. Additional suitable materials used to construct certain embodiments of the substrates include, but are not limited to, poly-para-xylylenes (e.g., parylene, including but not limited to parylene A, parylene AM, parylene C, ammonia treated parylene, parylene C treated with polydopamine), poly(lactic acid) (PLA), polyethylene-vinyl acetate, poly(lactic-co-glycolic acid) (PLGA), poly(D,L-lactide), poly(D,L-lactide-co-trimethylene carbonate), collagen, heparinized collagen, denatured collagen, modified collaged (e.g., silicone with gelatin), other cell growth matrices (such as SYNTHEMAX™), poly (caprolactone), poly(glycolic acid), and/or other polymer, copolymers, or block co-polymers, poly(caprolactone) containing cyclic Arginine-Glycine-Asparagine, cyclic or linear Arginine-Glycine-aspartic acid, blends of polycaprolactone and polyethylene glycol (PCL-PEG), thermoplastic polyurethanes, silicone-modified polyether urethanes, poly(carbonate urethane), or polyimide. Thermoplastic polyurethanes are polymers or copolymers which may comprise aliphatic polyurethanes, aromatic polyurethanes, polyurethane hydrogel-forming materials, hydrophilic polyurethanes, or combinations thereof. Non-limiting examples include elasthane (poly (ether urethane)) such as Elasthane™ 80A, Lubrizol, Tecophilic™, Pellethane™, Carbothane™, Tecothane™, Tecoplast™, and Estane™. Silicone-modified polyether urethanes may include Carbosil™ 20 or Pursil™ 20 80A, and the like. Poly(carbonate urethane) may include Bionate™ 80A or similar polymers.

Substrate Fabrication and Manipulation

Depending on the materials selected and the type of substrate to be fabricated (e.g., 3-D substrate cage or asymmetric inhomogeneous substrate), various techniques may be used to fabricate the devices. It shall be appreciated that the procedures listed herein are not exhaustive not meant to be interpreted as an exclusive list. Additional techniques known in the art, but not expressly disclosed herein may also be used to fabricate several embodiments of the invention. It shall also be appreciated that several embodiments of the substrates disclosed herein comprise combinations of materials, and therefore combinations of fabrication techniques may be used.

In several embodiments, the substrate is fabricated by extrusion, drawing, injection molding, sintering, micro machining, laser machining, and/or electrical discharge machining, or any combination thereof. In some embodiments, 3-dimensional substrates are fabricated as a single piece, however in several embodiments, the substrate is fabricated modularly. For example, in one embodiment, the top and bottom portions of a 3-dimensional substrate cage are fabricated independently of one another. Such an approach is advantageous in the production of substrates that differ from one another in one or more aspects (e.g., a first substrate has porous apical and basal surfaces and a second has a non-porous apical surface), but retain the same overall dimension. In such embodiments, the desired modular pieces may be selected and then assembled into a complete 3-dimensional substrate cage. After the desired modular pieces have been selected, they are aligned and sealed to create a 3-dimensional substrate cage with the desired dimension and characteristics. In some embodiments, a 3-dimensional substrate cage fabricated from a single piece is also sealed. For example, in one embodiment, the top and bottom portions are formed as a single flat piece and then one portion is folded over the other and the resultant 3-dimensional substrate cage is sealed. Sealing may be accomplished with heat welding, annealing, biocompatible adhesives or epoxies, and the like. Some embodiments optionally include a shell or frame comprising additional material that does not function as a cell growth surface. In some embodiments, the shell or frame provides a user one or more places to grip and or manipulate the substrate without damaging the cell growth surface and/or the cells growing on said surface.

While 3-dimensional substrate cages that function to foster interaction between the cells and the target tissue while retaining cells in the cage post-implantation are preferred in several embodiments, as discussed above, several embodiments comprise a substrate material (e.g., a biodegradable material) having at least one homogeneous cell growth surface and additional features for structural support. Some such embodiments are advantageous in that their production may be simplified as compared to a 3-dimensional substrate cage.

Moreover, in several embodiments, substrates with one homogeneous apical cell growth surface are suitable for positioning in a target site in a manner that still retains the cells on the homogeneous (apical) surface of the substrate and facilitates the therapeutic interaction with the target tissue. In other words, the lack of a 3-dimensional or cage-like structure does not reduce the therapeutic efficacy of substrates as disclosed herein.

In several embodiments, manufacturing of a plurality of substrates (or the modular portions thereof) is accomplished simultaneously. In some embodiments, the cells to be used for therapeutic effect are grown on the material that comprises the substrate (or a portion thereof) prior to the final fabrication of the substrate. In some embodiments, the substrates (or modular portions thereof) are effectively used as an in vitro culture substrate for the cells and at an appropriate time, are processed through the final phases of fabrication (if any are needed, depending on the embodiment) and ready for in vivo implantation with the cells pre-loaded in or on the substrate. For example, in one embodiment, a large sheet of polymer material is used as a substrate to grow a plurality of cells to be delivered in controlled laboratory conditions. When the cells are determined to be in an optimal state for delivery (e.g., a certain phase of cell cycle or a certain population density), a plurality of individual substrates are removed from the polymer sheet, sealed and ready to be implanted.

Several embodiments with homogeneous apical surfaces are particularly amenable to seeding and growth of cells simultaneously on a plurality of substrates, which are joined during the culturing process and are separated into individual substrates prior to insertion or implantation. For example, as shown generally in FIGS. **18**A-**18**C, multiple substrates can be fabricated from one contiguous piece of the chosen material (e.g., a biodegradable polymer; hereafter referred to as the frame). As shown in FIG. **18**A, a single circular frame is capable of providing mechanical support for multiple individual implantable substrates. In several embodiments, each substrate is attached to the larger frame via an extension (e.g., a handle; see generally FIGS. **18**B and **18**C). Dimensions of the substrates and handles are described in greater detail above. In several embodiments the handle of the substrate is free of cellular growth, allowing for manipulation and loading onto a surgical tool without disrupting monolayer integrity.

In several embodiments, the frame is dimensioned to maximize the surface area available for growth of cells seeded onto the substrate. For example, the frame may optionally be made in a rectangular shape and be comprised of a plurality of rectangular substrates. In one embodiment the rectangular substrates plus frame taken together maximize polymer to exposed culture dish surface area ratio by employing an interdigitating “comb-like” structure (see e.g. FIG. **18**D). It shall be appreciated that this figure is merely representative and a greater or lesser number of implants can be used, depending on the embodiment. In several embodiments a circular frame is preferred, as such frames can be dimensioned to fit within a standard cell culture dish (e.g., a 10 cm dish). In such embodiments, the close juxtaposition of the edges of the frame with the walls of the culture dish reduce flow and turbulence of the culture media over the cell growth surface, which can disrupt the integrity of certain growing cell populations. In some embodiments, larger or smaller diameters of substrate frame are used (e.g., diameters between about 5 cm to about 10 cm, from about 6 cm to about 9 cm, from about 7 cm to about 8 cm, and overlapping ranges thereof (frames may also be sized to fit in any variety of culture vessel as described herein).

As discussed herein, the plurality of individual substrates can be fabricated from a single, larger substrate frame using photolithographic techniques, injection molding, and the like.

Also as discussed herein, the individual substrates may be, without limitation, circular, oblong, or any shape customized to a specific to individual patient pathology (see e.g., FIGS. **5**-**8**). Customization of substrates is described in more detail below, by can be determined by electrophysiological testing (e.g. mfERG), psychophysical testing (e.g. kinetic or static microperimetery), or various ocular imaging modalities (i.e. spectral-domain optical coherence tomography (SD-OCT), fundus photography, fundus autofluoresence (FAF), or confocal scanning laser ophthalmoscopy (cSLO).

In several embodiments, the substrate frame is not designed to necessarily optimize or maximize a contiguous cell-growth surface, but rather is configured to allow selection and removal of a single substrate without disturbing other substrates on which cells are still growing. As shown generally in FIG. **22**A, in some embodiments, a plurality of substrates **10'** are fabricated on a single circular frame **130** positioned in a manner which allows the handle **30'** of the substrate to be accessed by a manipulation tool (e.g., forceps). Once fabricated, a single circular substrate can then be seeded with cells (e.g., H9 hESC-RPE) and cultured until such time that the cells have reached an optimal growth state (e.g., a confluent monolayer over the substrate). Individual oblong substrates are then cut from the larger frame using a sterilized and autoclaved pair of scissors or a custom designed holding/cutting tool. In several embodiments, each substrate comprises an identifier **155'** to aid the surgeon in orienting the substrate. In some embodiments, the identifier comprises a visual or chemical indicator (e.g., a fluorescent dye that can be visualized). In some embodiments, a MEMS reporting system is employed, and can report the viability of the cells or other local environmental conditions around the substrate. In several embodiments, a metal boundary or point is embedded into the substrate. Suitable metals include, but are not limited to nitinol, titanium, gold, silver, platinum, other metals and alloys, foils made from the same, and the like. In some embodiments, the substrates are doped with a fluorophore for imaging the substrate. In such embodiments, the loss or migration of cells from the substrate could be identified post-implantation or post-operatively, thus providing a means to assess the quality of the implantation procedure and to determine whether an alternate or additional substrate should be implanted. In some embodiments, the substrates further comprise a bar code or other unique identifier for quality control lot/batch information and inventory purposes.

A non-limiting example of a custom holding/cutting tool **140** is shown in FIG. **22**B. The cutting tool in fact functions in multiple additional ways, beyond simply enabling the cutting of an individual substrate from the frame. For example, in several embodiments the tool functions as a weight that holds the substrate frame in position within a culture vessel. In several embodiments, the holder/cutter device is reversibly attached to the underside of a tissue culture dish. In several embodiments, the holder/cutter device is integrated into the underside of a custom tissue culture dish. In such embodiments, the holder/cutter is advantageously pre-sterilized. In some embodiments, this is particularly advantageous, as some cell types require a greater media volume, which increases the flow of media throughout the vessel during normal handling. Such fluid flow could disrupt the integrity of growing cell populations, thereby increasing the overall time to prepare an substrate for implantation and/or adversely affecting the viability of the cells on the substrate. Further, the tool allows for the consistent and repeatable cutting of individual substrates.

Moreover, the tool, as it has a plurality of contact points **150** with the frame, prevents the growth of cells on a portion of the handle of the substrate. This cell-free portion is the portion which is grasped by manipulating tools (e.g., forceps) that are used in some embodiments. In such embodiments, the use of forceps does not disrupt the cells growing on the substrate growth surface, thereby maintaining the integrity and viability of the cells during the transition from the culture vessel to the target site of a subject. Each contact point also comprises a slot or aperture **151** that is dimensioned to allow a cutting device (e.g., a scalpel or custom designed sterilizable blade) to be inserted through the contact point and cut the underlying substrate handle, thereby freeing the individual substrate from the frame. The associated cutting point on the substrate frame is shown as **151'** in FIG. **22**A.

In some embodiments, substrates that are cut from the frame are manipulated and implanted with a specialized tool, which is described in more detail below.

Arrangements comprising a plurality of substrates within a frame are advantageous in some embodiments, because of the plurality of substrates, a certain number substrates may be used for release testing of the lot (e.g., assays testing genotype, phenotype, and function), which consists of one or multiple circular frames, while another portion of the substrates can be retained for implantation in a subject. Moreover, such a layout enables evaluation of each of the substrates in the frame for identification of the substrate that best suits a particular implantation procedure (e.g., has appropriate cells numbers, cell density, etc.) and subsequent selection of that substrate, without perturbation of the other substrates in the frame.

Fabrication of a plurality of substrates with cells pre-grown on the material provides certain additional advantages in some embodiments. In one embodiment, the possibility of contamination of the cells is minimized because the cells (and the material they are grown on) are already in sterile culture conditions and manipulation prior to implantation is limited.

Moreover, in some embodiments, growth of cells on a plurality of substrates allows the selection of the most healthy and viable cell populations prior to implantation. Additionally, those cells that are not optimal at the time of evaluation need not be discarded, but can be cultured for a longer period of time and/or under different conditions, until such time as they are optimal for implantation. In some embodiments a single point of connection between each substrate and the larger frame aids in simple cutting or dislocation of the substrate prior to surgery, further minimizing possible contamination of or damage to cells compared with similar designs requiring the full stamping-out or cutting of the substrate along it's entire perimeter (which may compromise the health and/or integrity of one or more cells on the substrate periphery). Furthermore, scale-up in manufacturing of a plurality of substrates with cells pre-growing is easily accomplished.

In addition to the methods used in the fabrication of the substrate, in some embodiments, additional processes are employed to further adapt the substrate, or the materials the substrate is fabricated from, to the particular cells to be used or target tissue. For example, in one embodiment, a polymer substrate, such as parylene, is used to fabricate the substrate. In one embodiment, the material surface that will form the cell substrate of the fabricated substrate is hydrophilically modified using oxygen treatment. This hydrophilic treatment generates an ideal surface for certain cell types to grow on.

In some embodiments, oxygen plasma treatment is performed using a reactive ion etch (ME). The etch is performed for two minutes at a power of 100 W and a maintained $O_2$ chamber pressure of 200 mTorr. In several embodiments, surface modification of parylene-c allows for the surface to remain hydrophilic for an extended period of time compared with other commonly used biocompatible polymers. $O_2$ plasma treatment will allow, in some embodiments, for maximal density of seeded cells, thus increasing the effective dose of the therapeutic to the targeted area.

For example, retinal pigmented epithelial cells (RPE) grow particularly well on a hydrophilic surface, as the RPE cells are polarized and thus orient themselves with respect to the hydrophilic surface. In several embodiments, the substrate enhances the ability of cells seeded thereon to polarize, thus reducing the likelihood of shedding or migration off the substrate during the delivery process.

In several embodiments, one or more surfaces of the substrate are altered to enhance handling and/or visualization of the substrate. For example, the outer surfaces of the substrate may be etched in order to roughen the surface. The rougher surface, in some embodiments, provides a surface which diffracts light to a greater degree than a smooth substrate surface, which reflects light. In some embodiments, this diffraction of light allows a user to visualize the substrate and/or tissues underneath or distal to the substrate more clearly.

MEMS Features

As discussed above, in several embodiments, the substrate are MEMS devices and/or incorporate MEMS technology. In several embodiments, the substrate comprises a central unit for data processing, one or more microsensors that evaluate the cellular environment within the device and or surrounding the target tissue. In several embodiments, the substrate further comprises a reporting unit that indicates certain information about the environment surrounding the substrate or the cells within the substrate. In some embodiments, the substrate reports on the viability or metabolic condition of the cells within the substrate. In some embodiments electrodes can be used to report the electrical impedance value at the device-tissue interface, and quantify proximity and/or mechanical force and pressure. These electrodes are also used, in some embodiments, to measure oxygen concentration and/or to measure blood flow. In some embodiments, the substrate reports on the degree of interaction between the cells within the substrate and those of the target tissue. For example, in certain ocular embodiments, the device reports to a user information regarding the degree of physical interaction taking place between the RPE cells within the device and target photoreceptor cells. In some embodiments, Fast CV is used to monitor the basal concentrations of electroactive species (as discussed above) to assess the health of the cells and tissues. This is advantageous in some embodiments, because photo-oxidative stress is a known cause of AMD and the device would be implanted close enough to the choroid to measure rate of blood flow. Moreover, in several embodiments, the device allows for assessment of underlying cellular morphology and health through the use of advanced imaging tools (e.g., spectral-domain optical coherence tomography (SD-OCT), fluorescein angiography (FA), fundus photography, SD-OCT supplemented with adaptive optics (AO-SD-OCT). In one embodiment, the boundary between RPE cells within the device and the endogenous photoreceptors (the PR-RPE-choriocapillaris boundary) is visible using long-wavelength SD-OCT imaging, thereby enabling adequate assessment of the ability of the cells to restore function.

In several embodiments, the substrate is configured to electrochemically detect electroactive species (e.g., neurotransmitter concentration, such as noradrenalin (norepinephrine), adrenaline, serotonin and dopamine). In some embodiments, fast cyclic voltammetry, proximity to tissue measurement using impedance, or other similar electrochemical detection methods are used to allow the device to report on the presence and/or concentration of electroactive species.

In several embodiments, the features of the substrate described herein facilitate the use of imaging techniques. In particular, ocular imaging techniques are used in some embodiments. In some ocular-directed embodiments, for example, the features of the device allow the assessment of the health of endogenous RPE and PR. Optical coherence tomography (OCT) is used in some embodiments and allows non-invasive imaging (e.g., without injection of dyes or radioactive labels) with a high degree of resolution. Such techniques allow for the imaging of about 10-100 cells in an intact eye. Moreover, advanced imaging techniques allow for the assessment of blood flow dynamics at the capillary level that supports RPE cells (choriocapillaris), and also the assessment of bleaching and rejuvenation of photoreceptor visual pigments; both of these are important metrics of the functionality of the RPE-PR complex.

Advantageously, many of the materials used in traditional MEMS devices are those described herein as being suitable for fabrication of the substrate. For example, the MEMS components of the device may be fabricated from, for example, polymers, silicon, or various metals. In those embodiments in which polymer-based MEMS devices are fabricated, processes such as injection molding, embossing or stereolithography may be used. In those embodiments in which silicone-based MEMS devices are fabricated, procedures such as deposition of material layers, patterning of the layers by photolithography, and then etching to produce the required shapes. In those embodiments in which metal-based MEMS devices are fabricated, procedures such as electroplating, evaporation, and/or sputtering may be used. Other processes such as molding and plating, wet etching or dry etching, electro discharge machining (EDM), and other similar processes known in the art are used in several embodiments.

In several embodiments, MEMS features are used to anchor a device to a target tissue. In some embodiments, MEMS latches or clamps are used to grasp the surface of a target tissue.

Custom Fabrication

In several embodiments, substrates are custom fabricated depending on the disease to be treated and the particular characteristics or symptoms of the individual afflicted with the disease. In several embodiments, a physician will make a determination regarding the distribution of damaged or diseased cells in particular patient. As a result, a customized substrate is generated that places the substrate and the cells associated with the substrate in the regions correlating to the damaged or disease cells.

For example, in ocular applications, the visual field of a patient can be determined by any appropriate known diagnostic techniques, such as Goldmann kinetic perimetry (see, e.g., FIG. **5**). Thereafter (or in place of), local visual function can be determined by any appropriate known technique, to generate a multi-focus electroretinogram (see, e.g., FIG. **6**). With this data, a custom substrate can be fabricated that places cells in juxtaposition to the area of lost or diminished visual function, that area corresponding to dead or functionally damaged photoreceptors.

In several embodiments, an injection mold is formed based on the determination of an individual's visual field/visual function (see e.g., FIG. **7**). In some embodiments, the mold comprises plastic, aluminum, or other easily workable (e.g., machinable) materials. In some embodiments, the mold can be stamped or shapes from elastomeric materials in a photographic process flow. In several embodiments, the materials are spin coated onto larger discs of material prior to stamping, thereby generating more uniform thicknesses in the resultant stamped substrate. In some embodiments, the mold form corresponds to the apical or basal surface of the substrate. The material of choice for the substrate, for example parylene (or other suitable material described herein) is shaped to the mold. In one embodiment, a solution of parylene polymer molecules is deposited into the mold and UV cured. The apical and basal surfaces are thereafter aligned and sealed, as described herein. In several embodiments, a metal trace is deposited on the apical and basal surfaces of the substrate (e.g. around the perimeter) to enhance alignment and sealing of the two portions of the substrate (see element **90** in FIG. **8**). In some embodiments, a platinum trace is deposited on the substrate halves by electron beam evaporation followed by electrochemical platinum deposition. In some embodiments, an Indian-tin oxide is deposited with sealing of the halves accomplished by flip-chip bonding. In several embodiments, MEMS latches are used to attached the various substrate components to one another. Other metal traces and bonding techniques known in the art are used in other embodiments. Not only does this metal trace provide enhanced alignment of the halves of the substrate, it also optionally provides additional thickness (depending on the amount of metal deposited) and adds structural support to the substrate (e.g., to prevent folding or crimping during the implantation process).

In several embodiments, the cells housed in such a substrate cage for ocular therapy are RPE cells. It shall be appreciated that RPE cells are also used in other non-cage embodiments. Substrates in accordance with several embodiments described herein are particularly advantageous for use in ocular applications, as the function of damaged photoreceptors can be supported (e.g., function is regained or further loss of function is prevented and/or minimized) by a physical interaction between the RPE cells retained within the substrate and the photoreceptors; RPE engraftment is not required.

In several embodiments, the RPE stem cells are modified to secrete neurotrophic factors that further support survival of photoreceptor cells. In certain such embodiments, genes encoding one or more neurotrophic factors are cloned into the RPE cells prior to transplantation such that there is significant and enhanced protection afforded to the photoreceptor neurons. Examples of such possible factors include PEDF, CNTF, BDNF. Over expression of other genes may allow for enhanced function of RPE. Examples include: over expression of surface integrins (which have been shown to increase RPE adhesion to BM), melanin (ethnic groups with increased melanin production are at lower risk for AMD), or receptors required for phagocytosis of shed discs (e.g. CD36, MerTK). In some embodiments, over expression of receptors required for phagocytosis is advantageous because at the time of therapeutic intervention there is likely to be an accumulation of lipofiscin and metabolic waste products that would require removal.

In several embodiments, the substrates and cells housed therein are employed in the treatment of age-related AMD, and in several preferred embodiments dry AMD. Other approaches for treatment of dry AMD include macular translocation and autologous RPE transplantation. Both are complex surgical procedures requiring general anesthesia and both are associated with high rates of retinal detachment. Moreover, there are no FDA-approved, effective pharmacologic therapies for dry AMD. Multivitamins only slow the progression of early AMD. With regard to other cell therapy approaches, differentiation of hESC or retinal progenitors into photorecptors is complex, as implanted photorecptors would have to integrate and form synapses with the host tissue. In contrast, the substrates disclosed herein allow the support of existing photoreceptors through the interaction between RPE cells within the substrate without the need for engraftment and/or synapse formation. Moreover, several embodiments of substrates disclosed herein promote the formation of a monolayer of cells within the substrate, which is advantageous as compared to a cell suspension. In some embodiments, a monolayer of cells on a substrate more closely mimics the structure of Bruch's membrane, which cannot, in AMD, provide a substrate for the attachment and further differentiation of injected cell suspensions. Thus, some embodiments of the substrates provide a synthetic replacement for the tissues damaged in AMD, rather than attempting to deliver cells that may not have an optimal tissue to attach to in vivo.

Cell Growth

In several embodiments, the substrate cages (and/or inhomogeneous substrates) are fabricated in a manner that provides an ideal surface and environment for the growth, survival, and function of cells. As discussed above, various characteristics of the substrates may be altered in order to surgical aspects of the substrate. To reiterate, the shape, thickness, pore diameter, and pore density are varied, in certain embodiments to optimally allow the housed cells to thrive and produce interactions with the target tissue, while being retained within (or on) the substrate. Thus, in some embodiments, the substrate confers the therapeutic benefit of the housed cells onto the target cells without the engraftment of the cells housed within the substrate. For example, in one embodiment, an epithelial layer of cells within the substrate (e.g., RPE) that are differentiated from hESC treat neurological degeneration (e.g., photoreceptor degeneration), without the need for synapse formation or neural integration. Moreover, these characteristics can be optimized to prevent migration of the cells out of (or off) the substrate and into the target tissue. Further, these characteristics may be varied in order to provide sufficient structure and resilience for the substrate to be implanted in vivo without damaging the substrate or the cells housed in the substrate.

As discussed above, the cell growth surface may also be altered (either before or after fabrication is complete) to optimize the surface for a particular cell type. In several embodiments, the cell surface is treated with oxygen to generate a hydrophilic cell growth surface on the substrate. In some embodiments, the oxygen treatment further comprises an additional compound, such as a matrigel, to support growth of polarized cells (e.g., RPE). In several embodiments, such matrigel compounds are xeno-free. Also as discussed above, in certain embodiments, the pore diameter may be adjusted depending on the cell type to be housed in the substrate.

In several embodiments, immunosuppressive agents are also delivered in order to avoid rejection of allogeneic cells introduced by the substrate. In some embodiments the substrate may be used to provide targeted and released delivery of these agents or drugs such as corticosteroids (e.g. prednisone), methotrexate, cyclosporine, antimetabolites, T-cell inhibitors, and alkylating agents. The administration of such immunosuppressive agents is used to avoid rejection of grafts or transplants or in treatment of autoimmune disorders. However, high-doses may be required, and oral administration, or even more localized and targeted delivery via injection, can be dangerous due to the increased risk of infection. In several embodiments, introduction of these agents via controlled and targeted release from substrates disclosed herein increases the likelihood of allogeneic graft acceptance, and minimize the risk of infection caused by immune suppression.

Other drugs that have been used clinical trials for treatment to AMD can be released by the substrate in a similar controlled and targeted manner by embedding in a bio-degradable portion or in the interconnect case itself. Examples include lipid-lowering statins, retinoids (e.g. Acitretin, Alitretinoin, Bexarotene, Etretinate Fenretinide, Isotretinoin, Tazarotene, Tretinoin), and anti-VEGF drugs.

As discussed above, in several embodiments a second substrate is annealed to the primary substrate. For example, in some embodiments, a biodegradable substrate is employed. In one embodiment, a biodegradable polycaprolactone (PCL) material compounded polyethylene glycol (PEG) is used. The polymerizing PCL-PEG material is spin-coated onto glass coverslips to make thin films of a desired thickness. Upon treatment with aqueous buffer or media, the water soluble PEG is washed away and creates pores in the film. As discussed above, biodegradable embodiments may be customized, and in one embodiment, the ratio of the PCL to PEG affects the degradation rate of the substrate. As the PEG is washed away to form pores, the pores increase the surface area of the polymer exposed to water and therefore increase the degradation rate. Through this approach both the porosity of the films as well as the degradation rate can be manipulated based on the amount of PEG added to the polymer blend. In some embodiments, polymers with cyclic amino acids (Arg-Gly-Asp; cRGD) are annealed to the polymer film, thereby providing a polymer surface that promotes cellular adhesion and growth by mimicking the extracellular matrix protein, fibronectin.

Cell Loading

In several embodiments, cells to be used in or on the delivered substrate are cultured on the substrate prior to final fabrication of the substrate. As discussed above, this provides several advantages with respect to minimizing the risk of contamination or damage to the cells or the substrate. However, in several embodiments, the cells are deployed into or on a completely fabricated and sterilized substrate. Substrates as described herein may be sterilized by gamma irradiation, ethylene oxide, autoclaving, UV sterilization, or other known procedures without degradation or damage. Such cell deployment is carried out under sterile cell culture or sterile surgical suite conditions. Such embodiments have the advantage, among others, that optimally healthy and robust cells can be selected and deposited into the substrate just prior to implantation. For example, in several embodiments, cells are loaded into a 3-D substrate cage by a surgeon while in the operating room. This methodology is advantageous because a surgeon could select from several varieties of substrates, depending on patient characteristics or other parameters, and then load optimally healthy cells into the substrate. In some embodiments, a vacuum pressure device functions to assist the surgeon on holding and filling the substrate cage with cells. In several embodiments, this is advantageous because the vacuum pressure that holds the substrate cage also allows the volume of cell delivery fluid (that is potentially larger than the volume of cells) to be removed from the substrate cage through the biological vias. In some embodiments, the substrate is loaded into a surgical introducer and then loaded with cells. In some embodiments, the substrate is loaded with cells, then placed in the surgical introducer. In several embodiments using asymmetrical inhomogeneous substrates, cells are pre-seeded and stably growing on the apical surface of the substrate, before selection and subsequent implantation of the implant by a surgeon.

With reference to FIGS. **2**A-**2**B, and FIG. **9**, some embodiments of the substrate comprise a cell retention feature **70**. Such a feature functions to provide a one-way passage for cells into the lumen **50** of the substrate cage. In several embodiments, the retention feature is custom shaped to provide an interlock with a pipette (or other device) **100** that delivers the cells into the lumen of the substrate cage. In several embodiments a resealable or "self-healing" membrane is used such that a pipette or needle can puncture through the membrane into the lumen for delivery of cells, but upon withdrawal of the pipette or needle, leaves no open orifice for cells to escape from. In some embodiments a viscoelastic is used. In some embodiments a biocompatible adhesive is used. In still other embodiments a one-way valve is used. Such a valve may comprises two or more flaps which open into the lumen upon advancement of a delivery pipette, which allows for the deposition of cells into the lumen. Upon removal of the pipette, the flaps return to their closed position, thereby retaining the deposited cells within the lumen. In some embodiments, the one way valve is formed such that a liquid tight seal is created to prevent backflow of cells, while in other embodiments, a fluid-tight seal is not formed.

Cell Types

Given the wide variety of diseases that induce cell damage or cell death, a wide variety of cell types can be housed within substrates described herein to achieve therapeutic effects. In some embodiments, cultured cells are used. In several embodiments, banked cells are used. In some embodiments, the cultured cells comprise stem cells. Stem cells are pluripotent cells capable of differentiating into a variety of different cell types. In some embodiments, embryonic stem cells are used, while in other embodiments, adult stem cells are used. In several embodiments, the embryonic stem cells are human embryonic stem cells. Embryonic stem cells, which are typically derived from an early stage embryo, have the potential to develop into any type of cell in the body. In some embodiments, H1, H7, H9, SHEF-1, or other similar FDA-approved stem cell lines are used. Adult stem cells are typically multipotent and can develop into a more limited number of cell types, typically those that are related to the tissue type from which the cells were isolated. In some embodiments, the stem cells are allogeneic to the recipient (e.g., as is the case with embryonic stem cells). In some embodiments, the stem cells are autologous to the recipient. In other embodiments, syngeneic cells are used, while in still other embodiments, xenogeneic cells are used. In some embodiments, freshly isolated cells are cultured and deployed into or onto the substrate for implantation into a recipient individual. In other embodiments, cryopreserved cells are used. In some embodiments, induced pluripotent stem cells are used.

In several embodiments, stem cells are isolated (or thawed) and cultured under standard sterile in vitro culture conditions. In some embodiments, the cells are cultured in standard tissue culture media known and used for the growth of stem cells. In other embodiments, a xeno-free culture media is used. In some embodiments, culture media is supplemented with one or more growth factors in order to support the viability of the cells and/or induce differentiation into a particular cell type. In some embodiments, growth factors such as are included in serum used to supplement the growth media. In some embodiments, specific factors are added, such as, for example, fibroblast growth factor, transforming growth factor (e.g., TGFβ1), insulin-like growth factor, e.g., IGF-1). Cells may be grown, passaged, and continually cultured until such time that the cells have optimal characteristics for deployment into the substrates described herein. At that point, the cells (if suspension cells) may simply be collected, adjusted to a desired cell density, and deployed (e.g., injected) into the substrate. Cells that are grown on a surface may be enzymatically digested (e.g., trypsinized) or mechanically detached from the surface, adjusted to a desired cell density, and deployed (e.g., injected) into the substrate.

Due to the features and advantages of several embodiments of the substrates described herein, in several embodiments, cell numbers that are deployed into or onto the substrate are relatively low. In several embodiments, the number of cells delivered ranges from about $1.0\times10^3$ to $1.0\times10^8$. In several embodiments cell numbers deployed into or onto the substrate range from about $1.0\times10^4$ to about $1.0\times10^7$, from about $1.0\times10^5$ to about $1.0\times10^6$, and overlapping ranges thereof. In one embodiment, about $1.5\times10^5$ cells are used. In other embodiments, greater or lesser numbers of cells are used.

As discussed above, cells from a variety of tissues may be used. In several embodiments, ocular cells are used to treat ocular diseases including, but not limited to age related macular degeneration (wet or dry), diabetic macular edema, idiopathic choroidal neovascularization, or high myopia macular degeneration. In some ocular embodiments, RPE cells are used. In several embodiments, cardiac stem cells are used to treat cardiovascular disorders such as myocardial infarction, ischemic cardiac tissue damage, congestive heart failure, aneurysm, atherosclerosis-induced events, cerebrovascular accident (stroke), and coronary artery disease. In several embodiments, liver stem cells are used to treat liver disease such as hepatitis, cirrhosis, cancer, and the like. Diseases in other tissues, such as the kidney, lung, pancreas, intestine, and neural tissues, among others, may be treated with the methods and devices disclosed herein. In some embodiments, harvested bone marrow stem cells may be used to repopulate hematopoietic cells that are reduced due to leukemias, cancers, or therapies that reduce blood cell counts.

Delivery Methods

Substrates in accordance with embodiments described herein may be delivered by various methods depending on the target tissue. Substrates may be delivered during an open surgical process. For example, during ocular surgery a substrate may be delivered to a region of the eye. In several embodiments, substrates are implanted in a specific delivery procedure. In some ocular embodiments, the substrates are delivered to the sub-retinal space. In some embodiments, an ab-interno procedure is used. In other embodiments, an ab-externo procedure is used. In some embodiments, a pars plana surgical approach is used for implantation. In other embodiments a trans-scleral approach is used for implantation. In several embodiments, substrates are attached (e.g., sutured, adhered) to a surface. In several embodiments, substrates are delivered endoscopically, via catheter-based methods, intravascularly, intramuscularly, stereotactically (e.g., for delivery of the substrate/cells to the brain or other neural tissue) or by other means known in the art for a particular target tissue. Depending on the substrate design and the target tissue, customized surgical tools are used to make the delivery of the substrates less traumatic, faster, or otherwise less risky or more beneficial to the subject.

In several embodiments, the surgical approach for ocular implantation is a pars plana approach. In some species, the pars plana lies approximately 3.5-4 mm away from cornea. In several embodiments, the substrate is delivered parallel to the posterior eye wall.

## EXAMPLES

Examples provided below are intended to be non-limiting embodiments of the invention.

Example 1

Determination of Pore Diameter that Retains RPE Cells

In the eye, a healthy Bruch's membrane functions as a molecular sieve that regulates the exchange of nutrients and metabolic wastes between the retina and the choroid. Based on the sub-retinal location of certain ocular-directed substrates disclosed herein, the porosity of the substrate would ideally simulate these functions of a healthy Bruch's membrane.

To investigate the pore diameter range that would allow for function, cell migration assays were performed. Circular parylene discs were manufactured with varying pore diameters (1, 2, 3, and 5 um) and placed on top of Transwell polyester (PET) cell culture inserts with 8 μm pores. Inserts were placed into 24-well culture plates. RPE cells were seeded on the parylene discs with serum free medium inside the PET cell culture inserts and medium containing 20 μg/ml recombinant human PDGF (as a chemoattractant) outside the inserts. After overnight culture, the PET insert membranes and the 24-well culture plates that held the inserts were both stained with hematoxylin. Results indicated that RPE cell bodies could migrate through 2, 3 and 5 μm diameter pores, but not through 1 μm pores. Thus it was concluded that pore sizes ranging from about 0.5 to about 1.5 μm are optimal for certain RPE-containing substrates.

Example 2

Biodegradable Polymer Substrates

As discussed above, parylene substrates are used in several embodiments. Biodegradable polycaprolactone (PCL) material compounded with polyethylene glycol (PEG) is used to test the formation of pores in substrates. During polymerization, the PCL-PEG material is spin-coated onto glass coverslips to make thin films of a desired thickness, as discussed above. Cyclic amino acids (Arg-Gly-Asp; cRGD) covalently bound to a PEG-PCL copolymer is annealed onto the surface of the films. Upon treatment with aqueous buffer or media, the water soluble PEG is washed away and creates pores in the film. The ratio of the PCL-PEG affects the degradation rate of the substrate. These pores increase the surface area of the polymer exposed to water and therefore increase the degradation rate. Through this approach both the porosity of the films as well as the degradation rate may be manipulated based on the amount of PEG added to the polymer blend.

Example 3

In Vitro Analyses of hESC-RPE

The present example demonstrates that hESC colonies grown to superconfluency, in the absence of FGF2, on mouse or human feeder layers or on matrigel, routinely produce discrete pigmented foci, which grow in size. These pigmented foci can be excised and expanded to produce highly differentiated monolayers that are similar to cultured fetal RPE or adult human cultured RPE. See FIG. **11**. Cells can be produced in quantities required for clinical use at 99% purity, as determined by microscopic analysis of pigmented cells as well as quantitative PCR for nanog and Oct-4, markers of possible contaminating undifferentiated hESC. Monolayer cultures are phenotypically stable after prolonged culture (11 months) without passage. Furthermore phenotype and normal karyotype can be maintained for up to at least 4 passages. Based on cell numbers in culture, some embodiments employ about $1.5\times10^5$ cells on a 5 mm diameter substrate are used for each treated eye.

Multiple RPE lines derived from different hESC lines have been characterized with respect to global RNA expression pattern, and quantitative analysis of key RPE marker mRNAs and proteins. Human ESC-RPE are remarkably similar to native human fetal RPE in their expression patterns (correlation coefficient of 0.97 between hESC-RPE and cultured fetal human RPE). Quantitative RT-PCR showed similar levels of RPE-specific transcripts important in gene regulation (Mit-F, OTX-2, Rax, Six-3), pigment synthesis (Tyr, Tryp-1, Tryp-2, Silver), retinol production (CRALBP, RPE65), tight junction formation (ZO-1, Claudin-3), and other key RPE markers (Bestrophin, Emmprin, Transthyretin, Pigment Epithelium Derived Factor (PEDF). hESC-RPE express proteins associated with these transcripts, as well as other crucial RPE markers (integrins, laminins, fibronectin, apoE, fibulin-5, pMe117. The expression of RPE65 by these cells is of particular significance, as deficiencies in this protein leads to blindness.

Like native RPE, hESC-RPE monolayers are polarized with apical microvilli, basal nuclei and endfeet, and lateral tight junctions, as detected by electron microscopic analysis and measurement of trans-epithelial resistance (TER). They carry out targeted membrane trafficking with apical localization of Na/K ATPase, integrin alphavbeta5 expression, apical secretion of PEDF, and basal secretion of collagen IV and VEGF. In addition, the apical surface of HESCRPE includes 1-2 cilia per cell that express a-acetylated tubulin, with a 9+0 structure typical of human fetal and early postnatal rodent RPE. Polarized sheets of hESC-RPE express tight junction proteins (occludin, claudin and Z01) localized to cell-cell contact regions typical of mature RPE cells, and generate TER typical of native RPE in situ. See FIG. **12**A.

To show that hESC-RPE cells are capable of efficient phagocytosis of photoreceptor outer segments (a process which occurs on a diurnal cycle in vivo and is required for vision), fluorescently tagged photoreceptor rod outer segments (ROS) were added to sheets of hESC-RPE, fetal RPE, or H2S7 fibroblasts (negative control) in vitro and binding/phagocytosis was quantified. See FIG. **12**B. hESC-RPE are quantitatively similar to fetal RPE in their phagocytic ability, and that activity was blocked by antibodies to alphavbeta5 integrin or MerTK receptors. Additionally, primary human retina (obtained from macular translocation surgery) were placed onto sheets of hESC-RPE, and phagocytosis of human photoreceptor outer segments was detected.

Additional function analyses are performed in some experiments. For example, in several embodiments isomerization is of all-trans-retinaldehyde to 11-cis-retinaldehyde is evaluated. The is a function performed by the RPE in situ which is important to the visual cycle and maintenance of vision. The ability of hESC-RPE to perform this enzymatic isomerization will be monitored by HPLC analysis of the conversion of all-trans-retinyl palmitate to the 11-cis form in aqueous cell homogenates. Another important function of RPE cells in situ is the phagocytic clearance of shed photoreceptor outer segment membrane. Phagocytosis is monitored using bovine rod photoreceptor outer segments labeled with SNAF1-2 (Molecular Probes) so as to allow differentiation between surface-bound and internalized outer segments. Pigment epithelial derived factor (PEDF) is a major secretory product of RPE cells in situ. It acts as an inhibitor of angiogenesis and has potent neuroprotective properties. Its secretion by cultured human fetal RPE and hESC-RPE is characteristic of establishment of a polarized epithelial monolayer with high trans epithelial resistance in several embodiments, therefore, the presence of PEDF in conditioned medium from hESC-RPE cells is monitored using an ELISA assay.

Example 4

Disease Modifying Activity of hESC-RPE in Animal Models of Retinal Dystrophy

hESC-RPE can rescue photoreceptors and visual function. hESC-RPE cells transplanted into dystrophic RCS rat retina survived in the subretinal space, continued their maturation in vivo, and phagocytosed ROS. Some approaches favored subretinal injection of hESC-RPE, our approach is to grow hESC-derived RPE on biocompatible substrates and implant these "patches" into the subretinal space in order to reestablish a functional RPE-photoreceptor interface. Although injected cell suspensions may be easier to perform, delivery of cells on a substrate that mimics Bruch's membrane is used in several embodiments. There are two main reasons for this change of approach. Firstly, the aged macula contains both aged Bruch's membrane as well as aged RPE cells, and this aged membrane does not support attachment and growth of RPE cells. Secondly, a large proportion of injected cells will ultimately be lost in the process due to mechanical, or apoptotic processes; a phenomenon that has been demonstrated in cell-suspension injection studies. In contrast, hESC-RPE grown on polylactic glycolic acid (PLGA; a biodegradable polymer); transplanted into the subretinal space of immunosuppressed RCS rats, can be precisely imaged by fundus photography and OCT. See FIG. **13**. In addition, high resolution imaging is performed and used to evaluate the placement and integrity of subretinal grafts. High speed spectral domain OCT technology (SDOCT), imaging allows for detailed three-dimensional imaging of rodent retina and visualization of various layers. See, for example, FIG. **16**.

The substrate is configured to degrade entirely within 90 days of implantation. Three weeks after transplantation, eyes were processed for pathologic examination. Immunostaining using the human specific marker TRA-1-85 revealed survival of the transplanted hESC-RPE cells in the subretinal space of dystrophic RCS rats and preservation of the overlying photoreceptors in the outer nuclear layer (ONL) compared to adjacent retina, control eyes or polymer-alone injections. See FIG. **14**.

Example 5

Treatment of Age Related Macular Degeneration

Age related macular degeneration is a condition found in elderly adults in which the macula area of the retina suffers thinning, atrophy and bleeding. This results in the loss of vision in the central area of vision, particularly an inability to see fine details. AMD is classified as either dry (non-neovascular) or wet (neovascular), with wet-AMD typically leads to more serious vision loss. Dry macular degeneration is diagnosed when yellowish spots known as drusen begin to accumulate from deposits or debris from deteriorating tissue primarily in the area of the macula. Gradual central vision loss may occur with dry macular degeneration. Dry AMD can progress to wet AMD, in which new blood vessels grow beneath the retina and leak blood and fluid. The leakage and pressure build-up damage light-sensitive retinal cells (photoreceptors), which either lose function or die, thereby leading to blind spots in central vision.

RPE transplantation strategies for AMD have been developed over the past 20 years, with the goal of re-establishing the critical interaction between the RPE and the photoreceptor. Advances have come from a large body of animal work and more recently a number of human trials using the patient's own cells. However, current approaches are hampered by complexities in surgical procedure and limitations in cell supply and quality. The most compelling evidence from over 260 homologous and autologous RPE transplants, mostly for neovascular AMD, comes from macular translocation studies. This complex and risky surgical procedure involves detaching and moving the retina so that the damaged macular area of the retina is put into contact with healthy RPE. Although not strictly RPE transplantation, it is functionally an autologous RPE transplantation.

Other strategies for autologous RPE transplantation have evolved from submacular RPE-choroid pedicle flap rotation to sub macular RPE-choroids-free graft transposition, submacular injection of suspension of RPE cells from the peripheral fundus, and currently, submacular insertion of RPE-choroid patch graft from the peripheral fundus. The latter technique involves harvesting an RPE-choroid patch graft from the periphery followed by insertion under the macula and is being used currently by several European groups.

Although these results are encouraging in that vision is restored in many cases, they point out the complexity of procedures that require two large incisions in the retina; the first to harvest and the second to implant. Such large retinal incisions (retinotomies) are associated with high risk of retinal detachment. The approach of using cell suspensions has the added drawback that unattached cells can easily escape into the vitreous cavity, leading to proliferation and scarring on the surface of the inner retina, resulting in complex and often irreparable retinal detachments (proliferative vitreoretinopathy (PVR).

RPE cells have also been transplanted into the brain in clinical trials for Parkinson's disease, since they have been shown to produce dopamine. Relevant to several embodiments disclosed herein, these studies did not detect any tumor formation by RPE. In adult humans, RPE do not replicate extensively, and reports of spontaneously occurring RPE-derived tumors are extremely rare.

Substrates as disclosed herein are used to deliver RPE cells to the sub-retinal space of subjects having AMD to treat the vision loss by facilitating interaction of the RPE cells with the photoreceptors, thereby supporting the photoreceptors and preventing additional loss of function or death of the photoreceptors.

In vivo studies in rat models of AMD are performed. Substrates seeded with RPE cells are delivered to treated animals and compared to diseased rats receiving sham (e.g. empty) substrates or sham operated normal rats.

Clinical follow-up and characterization is performed on both treated rats and controls. Clinical assessment is done using fundus angiography (FA) (to assess vasculature health), fundus photography, fundus autofluorescence (FAF), optokinetic nystagmus (OKN), electroretinography (ERG), and multifocal ERG (mfERG). Histopathologic assessment of animals is performed for evidence of local tumor/teratoma formation, maintenance of a differentiated RPE phenotype of the transplanted cells with lack of cell proliferation (immunohistochemistry using cell cycle specific antibodies), migration of cells away from the monolayer, cell loss or damage to adjacent photoreceptors or Bruch's membrane/choroid complex, and inflammation (macrophages, T-cells), or reaction to the substrate. Transplanted hESC-RPE will be identified by monitoring expression of a human marker protein (TRA-1-

85). The need for immunosuppression is evaluated by measuring expression of MHC Class I and II in vitro (with and without activation by interferon-gamma) and in vivo by immunohistochemistry. Treated rats exhibit improved clinical assessment with respect to one or more of the parameters measured.

Example 6

Generation of Substrates Containing Stem Cells

As discussed above, in several embodiments, hESC-RPE can be differentiated into polarized monolayers on biodegradable and non-biodegradable substrates In several embodiments, cells are pre-grown on a substrate that is eventually incorporated into a three dimensional substrate cage for implantation. A sheet of suitable substrate, such as a polycaprolactone and polyethylene glycol (PCL-PEG) co-polymer is polymerized at the desired thickness. While polymerizing, the co-polymer is optionally spin-coated on a coverslip to tailor the thickness. A cyclic amino acid containing PCL-PEG co-polymer is annealed to the polymerized substrate. Aqueous media is then added to dissolve the water soluble PEG and generate pores. The porous substrate is then sterilized. Stem cells are then cultured on the sterilized sheet to a desired cell density. One or more pieces of cell-supporting substrate are then cut from the sheet, aligned and annealed with one or more additional pieces of co-polymer to generate a cell-containing three-dimensional substrate cage suitable for direct implantation.

In several embodiments PLGA is used to form a biodegradable substrate. As shown in FIG. **15**A, hESC-RPE grown on PLGA show well developed apical microvilli after 3 weeks of culture. Other embodiments employ non-biodegradable material. hESC-RPE grown on surface-modified non-biodegradable parylene also show excellent attachment and development of apical microvilli (see inset of FIG. **15**B) within 48 hrs of culture. While in some embodiments cells are grown on a substrate prior to fabrication of the final substrate, in other embodiments, cells are deployed into a fully fabricated substrate prior to implantation.

Example 7

Generation of Substrates Configured to Receive Stem Cells

As discussed above, in several embodiments, substrate cages are fabricated that can later have one or more varieties of stem cells deployed within the substrate cages prior to implantation in a target tissue. Two molds are created that correspond to a top (apical) and bottom (basal) portion of the desired three-dimensional substrate cages. The molds are configured to yield an inner lumen upon the assembly of the two portions of the substrate cages and an access means to delivery stem cells to the lumen post-assembly. A polymer is polymerized within each portion of the mold. Upon polymerization, the polymer is removed from its mold and exposed to an aqueous media to generate pores within the substrate. The pores are configured to retain cells within the final substrate cage and still allow interaction (physical, chemical, or otherwise) between the cells a target tissue. The two polymeric portions are then aligned and annealed to generate a three-dimensional porous substrate cage that is suitable to receive stem cells and then be implanted into a recipient in order to generate a therapeutic effect in said recipient through the interaction of the cells and the target tissue.

Example 8

Interdigitation of hESC-RPE and Pr Outer Segments in Rat Eye

Using a parylene substrate comprising at least one planar cell-growth surface, interdigitation of H9 hESC-RPE with photoreceptor (PR) outer segments was demonstrated. The Royal College of Surgeons (RCS) rat is an established animal model for inherited retinal degeneration. The genetic defect in RCS rats causes the inability of the retinal pigment epithelium (RPE) to phagocytose shed photoreceptor outer segments. hESC-RPE cells were seeded onto parylene substrates and grown in accordance with the techniques described above. Once the RPE cells has grown to confluency, an substrate was implanted into the RCS rat eye, with the cell growth surface juxtaposed with the outer nuclear layer of the photoreceptors. Control substrates (no cells) were implanted into RCS rats for comparison.

FIG. **23**A shows a stitched fundus photo of implanted parylene substrate in the RCS rat eye. FIG. **23**B depicts hematoxylin and eosin (H & E) staining one week post-implant in RCS rat. The lower right panel of FIG. **23**B shows that hESC-RPE interdigitate with PR outer segments. The majority of the hESC-RPE are retained on the apical surface of the substrate. Unseeded control substrates show no such connectivity (**24**B lower left panel). FIG. **23**C shows a cross-section of isolated orbit of implanted rat (1 week post-implant). FIG. **23**D is a high resolution close-up of the parylene substrate seeded with hESC-RPE. Interdigitation and localization of RPE somas on substrate surface can be seen. See also FIG. **27**, which is a scanning electron microscopic image of hESC-RPE seeded on a parylene substrate.

Example 9

Interdigitation of hESC-RPE and Pr Outer Segments in Rat Eye

As discussed above, interdigitation of hESC-RPE cells with the outer segments of the photoreceptors allows the functional and metabolic interaction between the RPE cells and photoreceptors to take place. This interaction supports the viability of the photoreceptors and as discussed in the prior examples, leads to functional vision recovery. In this example, the long term engraftment and interdigitation of RPE cells in dystrophic rats was assessed by transmission electron microscopy.

As with the Examples above, substrates comprising RPE cells were implanted into the subretinal space of dystrophic RCS rats at postnatal day 29. The substrate use in this example was approximately 6.5 microns this, though as discussed above, other dimensions may be used in various embodiments. Animals were sacrificed on post-natal day 38 (9 days post-surgery) and 87 (58 days after surgery). No immunosuppressive therapy was administered to the rats.

As shown in FIG. **24**, at post-implant day 9 the RPE microvilli are localized near the outer segment disks. At post-implant day 58, shown in FIGS. **25** and **26**, interdigitation between the RPE microvilli and the outer segment disks can be seen. Thus, even at extended time-points, the RPE cells are viable and are functionally interdigitated with the photoreceptor outer segment. This data demonstrates that RPEs implanted according the methods disclosed herein, and using the substrates disclosed herein, provide long-term functional engraftment of RPEs in the eye.

Example 10

Implanted Cell-Seeded Substrates Restore Function

As discussed herein, delivery of cells to a target site requires not only that the cells actually reach the target site, but are viable and functional at the target site. Preferably, the duration of viability and functionality are sufficient to provide a noticeable therapeutic effect. H9 hESC-RPE were seeded onto a 0.3 micron thin film parylene substrate (with 6.5 micron (height) support features on the basal portion) as described herein. The cell-seeded substrate was surgically introduced into the sub-retinal space of a RCS rat in accordance with several embodiments above. As shown in FIGS. **28**A-**28**C, the parylene substrate can be identified by the white arrow. As discussed above, the design of the substrate provides support for the substrate itself, and additionally protects the cells during the implantation procedure. Moreover, the substrate is designed to allow the reciprocal exchange of nutrients between the RPE cells seeded on the substrate and the rich blood supply of the choroid. FIG. **28**A shows staining for TRA-1-85, which is a human antigen marker, which shows that the cells seeded on the substrate are present in an intact monolayer. The staining also confirms that the cells that make up the monolayer are of human origin. FIG. **28**B depicts staining for RPE65, which is a protein involved in the process of visual pigment regeneration in the PR cells. The presence of RPE65 shows not only that the implanted RPE cells are viable (at 2 months post-implantation), but also that they are functional and active in the normal processes associated with phototransduction. FIG. **28**C shows DAPI (a non-specific cellular marker) stain overlaying with the TRA-1-85 and REP65. FIGS. **29**A-**29**C show an additional series of immunofluorescent images from another experiment performed as described above. As discussed above, these images show that the monolayer of seeded RPE cells is both intact and functional 2 months post-implantation.

Additional experiments were performed in order to determine the degree of visual function restored following implantation of RPE-seeded substrates. RCS rats were received either a bolus injection of RPE cells or a cell-seeded parylene implant as disclosed herein at post-natal day 28-32. FIGS. **30** and **31** depict data collected from subsequent OKN testing. In the OKN testing, subjects (rats in these experiments) are placed in a chamber surrounded by video monitors that generate a pattern of black and white columns that move around the subject. The time that the subject spends tracking the visual image is indicative of the visual function of the subject (e.g., more tracking time is associated greater visual function). FIG. **30** depicts OKN data collected from normal, untreated transgenic blind mice, and transgenic blind mice treated with H9 hESC-RPE injected into the sub-retinal space in a cell suspension (no supporting substrate). As expected, normal rats had the greatest tracking time as compared to both treated and untreated blind rats (normal were tested only out to post-natal day 45). At day post-natal day 40, the treated and untreated blind rats did not significantly differ in their visual function. However, between post-natal day 40 and post-natal day 45, the treated rats maintained approximately the same visual function. In contrast, the untreated rats experienced the beginning of a decline in visual function, which continued through post-natal day 60. Between post-natal day 45 and post-natal day 60, the treated rats showed a small but insignificant decline in visual function, but still had discernibly improved function as compared to untreated rats.

FIG. **31** shows OKN data from normal, untreated transgenic blind mice, and transgenic blind mice treated with H9 hESC-RPE seeded onto a parylene substrate and implanted in the sub-retinal space. Again, in the early post-implantation period, the various groups were not significantly different in their tracking time. At post-natal day 45, the normal rats, as expected, had substantially greater tracking time as compared to untreated and treated rats. While the rats treated with substrates did not show a significant difference in visual function as compared to untreated rats, past post-natal day 45, the treated animals showed repeated, significant improvements in visual function. Not only does this data show a clear improvement in visual function as a result of cell-seeded substrate implantation show as compared to untreated animals, but the improvement is also markedly greater than when an RPE cell suspension is administered.

For example, the head tracking time at post-natal day 60 in mice treated with a bolus injection was approximately 12 seconds, while in contrast, the animals treated with a cell-seeded substrate tracked images visually for nearly 30 seconds. Moreover, the trend of increasing tracking time in the animals treated with a cell-seeded substrate suggests that greater improvement would be realized at later time points.

FIG. **32** is an additional depiction of the post-implantation RPE cells that have functionally interdigitated with the host photoreceptors and are metabolically active. As shown, the substrate is positioned at the bottom of the figure. Positioned on the substrate apical surface are the seeded RPE cells. Shown at the top portion of the figure are the resident photoreceptors. White arrow heads depict outer segments (being shed from the PR) that stain positive for rhodopsin. White arrows within the RPE layer depict phagosomes within the RPE that contain rhodopsin positive fragments of out segments. This demonstrates that the RPE cells are viable, stable and functionally active in that they are taking up the shed outer segments of the RPE, one of the normal functions of native RPE cells.

Thus, in several embodiments, the substrates disclosed herein not only provide a surface for the formation of a monolayer of seeded RPE cells, but protect the cells during implantation into the eye of a subject, and also support the viability of the cells post-implantation. The design of the substrate is such that nutrients can still reach the seeded RPE cells, but the substrate provides sufficient support to allow the cells to maintain a monolayer in vivo. Moreover, the continued viability of the cells contributes to the overall restoration of visual function, as the seeded RPE cells functionally replace the dead or damaged RPE cells, as evidenced by their uptake of shed outer segments from the PR cells.

Moreover, in several embodiments, specialized surgical methods to implant such substrates seeded with cells are used. These surgical procedures not only allow placement of a substrate that is specific to a particular subject, but also allow for the placement of one, two, or more substrates, depending on the severity of damage to the ocular tissue of the subject.

Additionally, substrates and methods as disclosed herein are useful for the treatment of a variety of outer retinal dystrophies. Not only are the substrates disclosed herein suitable for implantation into various places of the retina, their design which enables nutrients to reach the cells seeded thereon, the substrates are suitable for supporting the growth and function of a wide variety of cell types. By way of example only, substrates as disclosed herein could, in some embodiments, be manufactured to be seeded with photoreceptors and implanted in order to treat retinitis pigmentosa.

Various modifications and applications of embodiments of the invention may be performed, without departing from the true spirit or scope of the invention. Further, the disclosure herein of any particular feature, aspect, method, property, characteristic, quality, attribute, element, or the like in connection with an embodiment can be used in all other embodiments set forth herein. Method steps disclosed herein need not be performed in the order set forth. It should be understood that the invention is not limited to the embodiments set forth herein for purposes of exemplification, but is to be defined only by a reading of the appended claims, including the full range of equivalency to which each element thereof is entitled.

What is claimed is:

**1**. A substrate for cellular therapy to treat diseased or damaged ocular tissue, comprising:

a solid planar parylene substrate configured for implantation into a human eye, the substrate comprising

a long axis and a short axis; and

a distinct parylene apical surface for the growth of a population of human embryonic stem cell-derived RPE cells, and a distinct parylene basal surface;

wherein the apical surface has a thickness ranging from about 0.1 to about 5 microns allowing passage of molecules having a molecular weight of up to about 75 kilodaltons and has an integrally formed continuous raised lip positioned around the perimeter to reduce migration of human stem cell-derived RPE cells off the apical surface; and

wherein the basal surface comprises a plurality of supporting protrusions, the supporting protrusions being periodic, columnar in shape, and running longitudinally along the length of the long axis of the basal surface of the substrate and the basal surface is configured to prevent the growth of blood vessels through said basal surface to said apical surface.

**2**. The substrate of claim **1**, wherein the parylene of said apical surface is selected from the group consisting of parylene A, parylene AM, ammonia treated parylene, and parylene C.

**3**. The substrate of claim **1**, wherein the parylene of said inhomogeneous basal surface comprises a polymer selected from the group consisting of parylene A, parylene AM, ammonia treated parylene, and parylene C.

**4**. The substrate of claim **1**, wherein one or more of said apical surface and said inhomogeneous basal surface is treated with one or more of matrigel, poly-L-dopamine, vitronectin, or retronectin.

**5**. The substrate of claim **1**, wherein the height of said supporting protrusions ranges from about 1 μm to about 500 μm.

**6**. The substrate of claim **1**, wherein one or more of said parylene apical surface and said parylene inhomogeneous basal surface are oxygen-treated.

**7**. The substrate of claim **1**, wherein said continuous raised lip has a height ranging from about 10 to about 1000 microns.

**8**. The substrate of claim **1**, wherein said continuous raised lip has a width ranging from about 10 to about 1000 microns.

**9**. The substrate of claim **1**, wherein the long axis and the short axis of parylene substrate each range from about 0.3 mm to about 7 mm.

**10**. The substrate of claim **1**, wherein said parylene inhomogeneous basal surface is treated with vitronectin.

**11**. The substrate of claim **1**, wherein said parylene apical surface is treated with vitronectin.

**12**. The substrate of claim **1**, wherein the damaged or diseased ocular tissue comprises ocular tissue afflicted with an outer retinal degenerative disease is selected from the group consisting of dry AMD, wet AMD, Stargardt's disease, Leber's Congenital Ameurosis, and retinitis pigmentosa.

**13**. The substrate of claim **1**, wherein the substrate is suitable for implantation into the subretinal space of the eye of a subject.

**14**. The substrate claim **1**, wherein upon implantation into the human eye, RPE cells on said substrate functionally interdigitate with the outer segments of the photoreceptors of the eye.

**15**. A substrate for cellular therapy to treat diseased or damaged ocular tissue, comprising:

a solid planar parylene substrate configured for implantation into a human eye, the substrate comprising

a long axis and a short axis; and

a distinct parylene apical surface for the growth of a population of human embryonic stem cell-derived RPE cells, and a distinct parylene basal surface;

wherein the apical surface has a thickness ranging from about 0.1 to about 5 microns allowing passage of molecules having a molecular weight of up to about 75 kilodaltons; and

wherein the basal surface comprises a plurality of supporting protrusions, the supporting protrusions being periodic, columnar in shape, and running longitudinally along the length of the long axis of the basal surface of the substrate, the supporting protrusions providing structural rigidity along the long axis and permitting flexibility along the shorter axis, and the basal surface is configured to prevent the growth of blood vessels through said basal surface to said apical surface.

**16**. The substrate of claim **15**, wherein the parylene of said apical surface is selected from the group consisting of parylene A, parylene AM, ammonia treated parylene, and parylene C.

**17**. The substrate of claim **15**, wherein the parylene of said inhomogeneous basal surface comprises a polymer selected from the group consisting of parylene A, parylene AM, ammonia treated parylene, and parylene C.

**18**. The substrate of claim **15**, wherein one or more of said apical surface and said inhomogeneous basal surface is treated with one or more of matrigel, poly-L-dopamine, vitronectin, or retronectin.

**19**. The substrate of claim **15**, wherein the height of said supporting protrusions ranges from about 1 μm to about 500 μm.

**20**. The substrate of claim **15**, wherein the long axis and the short axis of parylene substrate each range from about 0.3 mm to about 7 mm.

**21**. The substrate of claim **15**, wherein said parylene inhomogeneous basal surface is treated with vitronectin.

**22**. The substrate of claim **15**, wherein said parylene apical surface is treated with vitronectin.

**23**. The substrate of claim **1**, wherein the damaged or diseased ocular tissue comprises ocular tissue afflicted with an outer retinal degenerative disease is selected from the group consisting of dry AMD, wet AMD, Stargardt's disease, Leber's Congenital Ameurosis, and retinitis pigmentosa.

**24**. The substrate of claim **15**, wherein the substrate is suitable for implantation into the subretinal space of the eye of a subject.

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